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PHYSIOLOGICAL STUDY OF THE SYMBIOTIC GERMINATION OF ORCHID SEEDS

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Introduction

In two previous papers (**6, 7**) I have presented evidence that germination of orchid seeds is dependent upon an available supply of organic matter. These papers emphasized the nutritional aspects of the problem, and also presented certain views with respect to the possible function of the fungus in the pure culture experiments made by BERNARD (**1**), BURGEFF (**4**), and others. All who have studied the germination of orchid seeds agree that there is something very unusual about the seeds of most orchids, for they cannot germinate when merely supplied with water and nutrient salts. According to BERNARD, BURGEFF, RAMSBOTTOM (**11**), and CONSTANTIN and MAGROU (**5**), normal germination is dependent on infection of the embryo by the appropriate fungus. Symbiosis is believed by these men to be obligative for the normal development of orchids.

Symbiosis in a broad sense means the living together of two dissimilar organisms. Under this definition, a very wide range of associations might be considered. The term as applied to the relation between the orchid and the orchid fungus includes the idea that the fungus is in some manner of benefit to the orchid plant, and, according to BERNARD, BURGEFF, and others, germination is normal-

ly induced only after the embryo becomes infected. The experiments first made by BERNARD and later by BURGEFF were so striking, and to many seemed so conclusive, that obligative symbiosis has been accepted as established for orchids.

According to BERNARD, BURGEFF, and RAMSBOTTOM, the embryo becomes infected, and provided that the infection is confined to the lower part of the embryo, germination will take place. The explanation offered is that the fungus increases the concentration of the cell sap, and this increase in concentration acts as a physical-chemical stimulus to germination. BERNARD suggests that this increase might be brought about by the digestion of starch within the embryo, this digestion being effected by enzymes excreted by the fungus. He recorded experiments in which germination was obtained by the use of higher concentrations of the nutrient solution. This nutrient contained an extract of the tubers of a certain orchid. In discussing these experiments, BERNARD did not emphasize the nutritional aspects of the problem, but stated that the higher concentration was a physical-chemical stimulus to growth, and that one might compare the action of the fungus with the growth stimulus imparted to an egg by the spermatozoid. Furthermore, he stated that the stimulus to growth induced by the high concentrations of organic substances is comparable with the influence exerted by chemical or other treatments in inducing the development of an unfertilized egg. BERNARD concluded that the stimulus acts to increase the formation of absorbing hairs, and with the increased concentration of sap there is likewise an increase in the intake of water. BURGEFF, while differing somewhat in an explanation of the function of the fungus, expresses the view that both fungus and orchid are benefited by the association.

The evidence presented by adherents of the symbiotic view of germination may be summarized as follows: (1) the roots of orchids are generally infected by a characteristic fungus which is considered non-injurious; (2) different genera of orchids may have different strains or species of this fungus; (3) seeds sown under pure culture on various culture media, especially those media containing starch or other insoluble organic matter, do not germinate except when the fungus is present; (4) germination apparently is induced by some

strains of the fungus and not by others; (5) there must be maintained a definite balance between fungus and host; (6) while it is recognized that germination of orchid seeds may be obtained by the use of sugars in the culture medium, germination under these conditions is abnormal, and not of course common in nature.

In my first paper (**6**) considerable evidence was presented relative to the use of sugars and plant extracts in inducing germination. In a second paper (**7**) I extended these studies to include seeds of various orchid genera, and brought out other salient facts relative to the subject of symbiosis in orchids. In addition to the experimental evidence on the nonsymbiotic germination of orchid seeds, I included a critical discussion of the evidence presented by BERNARD and BURGEFF on the necessity of the fungus. I admitted at that time that certain evidence presented by these two investigators was difficult of interpretation except on the basis that the fungus was essential. The validity of the fungus hypothesis was questioned very largely on the basis that BERNARD and BURGEFF had failed to take into consideration the effect of chemical changes in the external medium on germination. Both used organic matter in the culture medium. In some cases starch was used. In BERNARD'S experiments salep was used, which is really pulverized tubers of a certain orchid. This latter substance contains some starch, pentosans, some sugar, and other organic substances.

These facts are important because of the well recognized ability of fungi to secrete enzymes which are capable of digesting a variety of substances. There is still another possibility that was ignored by the proponents of the symbiosis hypothesis, which is that substances secreted by the fungus or produced on autolysis of the fungus may be involved in the germination. In my first paper I stated as a possible explanation of the favorable effect of the fungus, that "It is conceivable that the germination is induced not by any action of the fungus within the embryo, but by products produced externally by digestion or secreted by the fungus." I also stated that the evidence as regards the necessity of the fungus was not yet proved, and that considerable work was yet to be done before the validity of the fungus hypothesis could be proved or disproved.

Another phase of the problem was presented by BERNARD,

namely, the loss by the fungus of its capacity to induce germination after prolonged culture in the laboratory. I stated: "It is entirely possible that there has been no loss in the fungus, but at the time of inoculating the culture, the physiological state of the embryos was such as to resist or permit of infection. Those in which the infection was confined to the lower cells could still germinate despite the fungus. Those invaded to a greater extent would be killed. These and other experiments suggest that one of the causes of failure of germination is the parasitic character of the fungus."

In planning these experiments on the relation of the endophytic fungus to germination, the questions that arose directly from a consideration of the problems were these: Does the fungus change starch to sugar? Is there any secretion by the fungus? Are the embryos that continue to grow always invaded? Are the embryos ever killed by the fungus? Is there any relation between the time of infection and the degree of injury or rate of germination? Are the external changes induced by the organism sufficient to produce germination? Are there any other fungi capable of inducing germination? Is there a change in the "activity" of the fungus when grown under pure culture conditions? Is the fungus equally effective in inducing germination under conditions of varying concentration of starch? Why is germination of *Cattleya* not possible with the fungus from *Odontoglossum?* These questions are considered in the experiments which follow. The evidence presented by these and various other experiments leads to but one conclusion, which is that the fungus is not necessary for germination, at least for seeds of *Cattleya*.

Methods

Except where otherwise indicated, the culture methods were essentially those described in my first paper. For the most part the culture vessels were culture tubes 200 mm. × 20 mm. Whenever the hydrogen ion concentration was to be controlled, the tubes were of Pyrex glass. The nutrient solution used for the most part was solution B of my first paper, although in certain experiments the dibasic potassium phosphate K_2HPO_4 was replaced by the monobasic phosphate KH_2PO_4. The reason for this change was to afford a

slightly more favorable hydrogen ion concentration. Solution B unmodified has the following composition:

$Ca(NO_3)_2$	1 gm.
K_2HPO_4	0.25 gm.
$MgSO_4NH_2O$	0.25 gm.
$FePO_4$	0.05 gm.
$(NH_4)_2SO_4$	0.50 gm.
Distilled water	1000.00 cc.

To this nutrient solution was added agar at the rate of 1.75 gm. for each 100 cc., and sugar or starch as the experiment required. The culture medium was heated to "dissolve" the agar, and then 10 cc. of the solution added to each tube. The tubes were plugged with cotton, autoclaved at 15 pounds pressure for twenty minutes, and then sloped. Each tube was then capped with a glass vial to decrease the possibility of spores lodging on the cotton and growing through it or between the cotton stopper and the side of the tube.

The seeds were sterilized by the use of calcium hypochlorite (13), using the filtered solution obtained by shaking 10 gm. of calcium hypochlorite in 150 cc. of water. They were then placed in a small tube about 60 mm. × 6 mm., and the hypochlorite added. The tube was then shaken until each seed became moistened, and after five or ten minutes in this solution the seeds were sown by means of a looped platinum needle, employing the usual bacteriological technique to prevent contaminations. After a few minutes in the hypochlorite most of the seeds accumulate on the surface of the solution, and are planted by means of a platinum loop without any previous rinsing. All the cultures were maintained in a small glass compartment in the greenhouse. No attempt was made to maintain a constant temperature. In the summer the tubes were shaded from direct sunlight. In general the temperatures prevailing were 20°–30° in the daytime and somewhat lower at night, except for marked departures during the spring and fall, when the houses were not heated.

Hydrogen ion determinations were made of the culture media, both inoculated and uninoculated. For this purpose a definite quantity of either methyl red or brom thymol blue was added to

the culture solution. To distribute the indicator throughout the agar mass, the tube was heated or left standing over night, by which time the indicator was fairly well distributed. Generally the tube was heated. On cooling, the color was compared with standard buffer solutions containing a like quantity of the desired indicator. The turbidity of the agar was compensated by interposing another tube of agar between the buffer solution and the source of light. In some of the experiments it was essential to adjust the hydrogen ion concentration of the culture solution. This was accomplished by first making up the solution with agar. This was then autoclaved and to this was added 0.1 normal HCl until the desired reaction was obtained.

Sugar determinations of the culture media containing starch were made by extracting the culture medium with 95 per cent alcohol. To the culture tube 10 cc. of 95 per cent alcohol was added. This was then heated in the autoclave, and when the contents of the tube was liquefied it was poured into a beaker containing a volume of alcohol ten times that of the original tube content. The alcohol precipitated all starch and agar. The alcohol extract was then filtered from the residue, and the residue washed with alcohol. The alcoholic extract was then evaporated to dryness in a water bath, and this residue redissolved in 25 cc. water. Sugar was determined by the Walker-Munson method for glucose. When sucrose was used the procedure was the same, but the reducing sugar is expressed as invert sugar.

In making growth measurements of these minute objects, the diameter of the embryo or protocorm of the seedling was measured. Measurements were made by the use of a microscope, using an ocular micrometer. The diameter of the embryo is not an exact measure of growth, since the embryos of two different culture media may not be of the same shape. The embryos at first are somewhat oval, later they are spherical, and still later they may become somewhat top-shaped. It would be an extremely tedious task to determine the volume of the seedling, and from most of the data here recorded the volume may be calculated from the diameter, since the embryos measured were spherical or nearly spherical in shape. This is true except for those embryos having a diameter of less than

225 μ. Each average figure given for the diameter measurements in nearly all cases is the average obtained from measurements of fifty individual embryos.

In studying the infection of the embryo by the endophytic fungus, it was found essential to prepare the specimens for microscopic examination. For this purpose the embryos were fixed in a chromo-acetic fixative and carried through the preparatory stages for imbedding in paraffin. For the most part fixation was good. The imbedded material was sectioned generally about 15 μ in thickness and then stained with Haidenhain's iron-alum haematoxylin stain. Various other stains were used, but the latter proved excellent as a stain for the hyphae. In using this stain the material was first overstained and then destained. The fungus retained the stain more markedly than the host tissue and protoplasm; consequently differentiation was quite marked.

Isolation of fungus

The isolation of the orchid fungus gave considerable trouble, and various attempts to isolate it resulted only in the isolation of a *Fusarium* and other fungi, some of which were quite destructive to the embryos. One of the causes of this failure was that the isolation was attempted from portions of the root which appeared most heavily infected, and which generally were far removed from the root tip. It would appear from such results that in the older portions of the roots the orchid fungus is no longer alive, and that secondary infections have occurred. There is evidence for this also in the fact that complete disintegrations of the roots of *Cattleya* and of other orchids are commonly observed.

In selecting roots for isolation of the fungus, therefore, healthy appearing roots only were selected, which were not at all discolored, but had a glistening whitish appearance. From such roots freehand sections were made, beginning at the root tip and continuing back from the tip until infected cells were found in the section. From this newly infected root tissue the isolation was made. The roots were thoroughly cleaned in running water and by means of a soft brush. They were then immersed for a few minutes in a solution of calcium hypochlorite made by shaking 10 gm. of the hypochlorite

in 150 cc. of distilled water, then filtered, and afterwards rinsed in sterile distilled water. The roots were then momentarily flamed, and by means of a scalpel and forceps the outer velamen was peeled off. The root freed of velamen was again passed through a flame very quickly. By means of a sterilized razor blade, the root was then cut into sections about 1 mm. in length, and one or two of the sections placed on the culture medium in a Petri dish. The culture medium used was solution B+0.5 per cent starch. Out of sixty-five plates made, twenty showed no growth at all; *Fusarium* and a few other fungi appeared in about twenty-five plates; in the remainder was noted a rather slow and weak growing fungus which was isolated. These appeared microscopically and macroscopically to be identical. The plant from which the roots were obtained was a vigorous growing plant of *Cattleya Portia*.

Using a slightly different procedure, DIXON, working under my direction, isolated an organism resembling that of BERNARD, and which proved later to be identical with the fungus which I isolated.

Influence of fungus on germination

EXPERIMENT 1.—Having isolated a fungus which morphologically fitted the description given by BERNARD for the fungus of *Cattleya* (*Rhizoctonia repens*), it was first necessary to prove the fungus the correct one by inoculation experiments, and to determine its ability to induce germination. The criterion of the true fungus, as RAMSBOTTOM states, is that it will induce germination. Some preliminary experiments gave positive results, although the percentage of germination was small.

A more elaborate experiment was devised, therefore, to determine the ability of the fungus to induce germination in various nutrient media, and at the same time to determine whether the fungus would have any influence on the chemical composition of the nutrient medium. If the fungus digested the starch of the nutrient medium, changing it to sugar, then the necessary condition would be provided for germination, and this fact would need to be taken into consideration. The results are given in table I. Three different nutrient solutions were used, and to each was added 1.5 per cent agar and starch. The solutions used were solution B as previously

TABLE I

SEEDS SOWN SEPTEMBER 16, 1922; NOTES MADE APRIL 12, 1923

Culture no.	Date of inoculation	Percentage germination	Percentage dead	Average diameter (μ)	P_H of culture solution	Mg. of reducing sugar	General condition
	PFEFFER'S CULTURE MEDIUM						
PA 3	September 16	13	9.0	506	4.6	.....	Light green; one leaf produced
PA 4	September 16	9	20.0	447	4.6	95.3*	Light green; one leaf produced
PA 7	September 28	65	1.5	651	4.6	.....	Dark green; one and two leaves
PA 9	Not inoculated	0	100.0	179	7.0	.....	Brown and apparently dead
PA 11	Not inoculated	0	100.0	151	7.0	5.4*	Brown and apparently dead
PA 12	Not inoculated	0	100.0	165	7.0	.....	Brown and apparently dead
	SOLUTION B						
BA 4	September 16	0	90.0	317	4.0	170	Few with leaves dark green; others still alive, light green; others brown and apparently dead
BA 5	September 28	2	80.0	380	4.0		
BA 7	September 28	0	100.0	359	4.0		
BA 8	September 28	0	100.0	279	4.0		
BA 9	Not inoculated	0	100.0	172	6.0	.....	.....
BA 10	Not inoculated	0	100.0	161	6.0	.....	Brown and apparently dead
BA 11	Not inoculated	0	100.0	162	6.0	.....	.....
	SOLUTION D						
DA 2	September 16	37	1.5	648	.....	.....	.....
DA 3	September 16	60	4.0	767	4.8	181*	.....
DA 4	September 16	0	53.0	588	.....	.....	.....
DA 5	September 28	25	1.0	648	4.8	.....	.....
DA 6	September 28	18	2.0	641	.....	.....	.....
DA 7	September 28	0	33.0	581	4.8	.....	.....
DA 8	September 28	20	6.0	679	.....	.....	.....
DA 9	Not inoculated	0	100.0	150	6.3	0.08	Brown and dead
DA 9	Not inoculated	0	100.0	131	6.3	.....	.....

* Total amount of sugar in three tubes.

described, Pfeffer's solution, and a third designated solution D. The formulas for the two latter solutions are:

Pfeffer's solution		Solution D	
$Ca(NO_3)_2$	4 gm.	$CaCO_3$	2 gm.
K_2HPO_4	1 gm.	K_2HPO_4	1 gm.
$MgSO_47H_2O$	1 gm.	NaCl	1 gm.
KNO_3	1 gm.	$(NH_4)_2SO_4$	2 gm.
KCl	0.5 gm.	NH_4Cl	0.5 gm.
$FeCl_3$	0.040 gm.	Tap water	1 liter
Distilled water	5 liters	Potato starch	0.6 per cent
Soluble starch	0.5 per cent		

An examination of table I brings out a number of significant facts. The fungus induced germination, but in no case was 100 per cent germination attained at the time of taking the final observations. In cultures PA 3, 4, 7, and DA 2 to 8, a greater percentage of germination would have been obtained had the culture been left in the greenhouse a few weeks longer. The fungus isolated not only fitted the description given by BERNARD regarding its morphological characteristics, but likewise induced germination. In the absence of the fungus no germination occurred, and the growth made by the embryos was characteristically slight. From these and other experiments later described there is abundant evidence regarding the effectiveness of the fungus in inducing germination.

What is the explanation of the stimulating action of the fungus? As shown by table I, in the inoculated cultures the hydrogen ion concentrations for the PA cultures are equivalent to P_H 4.6, while in the uninoculated cultures the values are P_H 7.0. Likewise the values are P_H 4.0 and P_H 6.0 for the BA series, while for the DA series the values are P_H 4.8 for the inoculated cultures and P_H6.3 for the uninoculated. Here is evidence that the hydrogen ion concentration is markedly increased by the fungus, and this must be due to organic acids produced and excreted by the fungus.

It will be noticed that the glucose content for the inoculated cultures is considerable, while in the uninoculated cultures there is practically no sugar. The small amount present probably is due to reduction by dextrins which were not precipitated by the alcohol, or to slight hydrolysis of some of the dextrin on heating the tubes. This shows that the fungus has digested starch, converting it to

sugar, and in its metabolism some of its sugar is changed to some organic acid. Thus in cultures PA 3, 4, and 7 the total sugar found was equal to 95.3 mg., or 31.7 mg. per tube. The original amount of culture medium added was 10 cc. to each tube, so that on this basis the concentration would equal 0.3 per cent sugar. At the close of the experiment, however, due to evaporation, there was present only 6 cc. of the culture medium, so that the final concentration was equal to 0.5 per cent sugar.

In cultures BA 4, 5, 7, and 8 the total sugar was 170 mg., or 42.5 mg. per tube. On the basis of 10 cc. per tube the initial concentration of sugar would be 0.4 per cent, but on the basis of 6 cc. the final concentration was about 0.7 per cent sugar. In DA 4, 5, and 7 there was present about 60 mg. of sugar per tube. The concentration of sugar, therefore, was nearly twice that of cultures PA 3, 4, and 7. This probably is the reason of the greater growth in the DA series.

The studies made by NANZ in this laboratory (not yet published) show that at a hydrogen ion concentration of $P_H 6$ growth is slow, even though sugar is provided; but at a hydrogen ion concentration of $P_H 4.7$ to $P_H 5.2$ growth is very much accelerated. At the higher P_H value the embryos are yellowish or even whitish in color, but at the higher hydrogen ion concentration (lower P_H) the embryos become deep green. The presence of sugar and the favorable hydrogen ion concentration are altogether adequate to explain the germination, without ascribing it to any internal action of the fungus.

Another interesting aspect of the fungus relationship is developed by the data in table I. It may be noted that in Pfeffer's solution only a relatively low percentage of the embryos were killed. With but two exceptions this is likewise true for the DA cultures. On solution B, however, a rather high percentage of the embryos in the inoculated cultures were killed. These data suggest that the degree of infection of the embryo depends upon the nutrient solution used, which in turn affects the physiological state of the embryo.

Slides were made of material from all these cultures, following the methods previously described. Microscopic examination of serial slides of individual seedlings of the inoculated cultures of series PA revealed some interesting facts. Not all of the seedlings

were infected by the fungus; some were markedly infected and others but slightly so. In the latter case the fungus was confined to one or two cells at the base of the protocorm. The fact that not all the seedlings were infected is significant, for if some develop without being infected, then it is apparent that the stimulative effect of the fungus is not internal, but is due to chemical changes in the culture medium. Results of like character were obtained with material of the other series.

EXPERIMENT 2.—In this experiment the methods used were essentially the same as those of the preceding experiment. Solution B was used, modified by substituting KH_2PO_4 for K_2HPO_4, and 0.25 per cent soluble starch instead of 0.5 per cent. The KH_2PO_4 was used to give initially a more favorable hydrogen ion concentration. The lower concentration of starch was used because BURGEFF used concentrations close to this value in some of his experiments. These cultures were kept in the laboratory three or four days before they were taken to the greenhouse. Detailed data are given in table II. These data indicate that the fungus was favorable to the germination of some of the seeds, but most of them were killed. A few of those living had produced leaves, but not all were green, indicating the pathogenic character of the fungus. Ninety per cent of the seeds were killed in the inoculated cultures, while in the control cultures the embryos were still living, but of course had made but little growth. Furthermore, in the cultures RA 11, 12, 15, and 16 the large living embryos or seedlings were infected but slightly, while most of the smaller embryos which had been killed were completely invaded. The explanation for the high mortality must be ascribed to the low concentration of starch and a consequently low concentration of sugar.

EXPERIMENT 3.—In view of the fact that so many seeds were killed by the fungus in experiment 2, and since the results were so unsatisfactory from the standpoint of favorable germination, another similar experiment was made. Some of the evidence in table I indicating that less injury resulted if the cultures were inoculated some days after sowing, it was decided to inoculate the cultures some time after the date of planting. One series was inoculated a week after planting, while the second series was inocu-

lated nearly six weeks after planting. The observation made by BERNARD and BURGEFF that the fungus produced a marked acceleration in growth of the embryo was noted in this and other experiments. The data appear in table III.

TABLE II

Cattleya HYBRID SEEDS PLANTED AND CULTURES INOCULATED DECEMBER 21, 1922; NOTES MADE JUNE 10, 1923

CULTURE NO.	PERCENTAGE DEAD	AVERAGE DIAMETER (μ)	OTHER OBSERVATIONS	P_H OF CULTURE SOLUTION	GLUCOSE PER TUBE (MG.)
	Solution B+¼ starch				
RA 1	0	190	All green	5.6	1.2
RA 3	0	193	All green	5.6	0.8
RA 7	0	190	All green	5.6	0.8
	Solution B+¼ starch inoculated				
RA 15	90	315	5 per cent green, 5 per cent albino	4.2	9.4
RA 11	90	432	5 per cent green, 5 per cent albino	4.2	7.4
RA 12	80	423	10 per cent green, 10 per cent albino	4.2	8.8
RA 16	85	432	6 per cent green, 9 per cent albino	4.2	7.2
RA 17	95	433	5 per cent green	4.2	7.5

TABLE III

Cattleya HYBRID SEEDS PLANTED MARCH 15, 1923; CULTURE SOLUTION B+¼ PER CENT STARCH

DATE OF INOCULATION	AVERAGE DIAMETER OF EMBRYOS (μ)			OBSERVATIONS MADE JUNE 15
	April 12	May 16	June 15	
Not inoculated	126	162	180	Light green; 3 per cent dead
March 22	171	243	288	Dark green; 3 per cent dead
April 27	.....	.....	252	Dark green; 3 per cent dead

On October 3 additional notes were taken on this series of cultures. Those cultures inoculated on March 22 had embryos which averaged 315 μ in diameter, while those inoculated on April 27 were 414 μ in diameter. Those of the control series averaged only 220 μ in diameter. In each of the inoculated cultures, 10 to 15 per cent of the embryos had developed to a well defined seedling stage,

each seedling having one or two leaves. The remainder were just about to produce the "leaf point." On this same date the culture solutions were analyzed for sugar, and hydrogen ion concentrations made. The control cultures (not inoculated) gave readings of P_H 5.7, while the inoculated cultures were P_H 4.4. Starch tests were likewise made. All the starch had been digested in the inoculated cultures, while in the uninoculated cultures starch had not been changed. Sugar determinations were also made. The inoculated cultures yielded 2–2.4 mg. glucose, while the uninoculated cultures yielded 0.3 mg. sugar.

The data in the table and those obtained on October 3 show the favorable effect of the fungus on germination. In this experiment, however, there was no killing of the seeds. This is due either to the fact that the cultures were inoculated some time after planting, or to inherent resistance on the part of the seed to the fungus, and perhaps also to the fact that the cultures were taken immediately to a well lighted greenhouse in March.

The fact that all the starch was digested by the fungus, and that sugar was produced, together with the production of a favorable hydrogen ion concentration, constitutes evidence that external changes have taken place, and, as in the preceding experiment, these changes are sufficient to cause germination.

The objection might be made that the amount of sugar in these cultures is too little to be of much significance, but it should be taken into consideration that these analyses were made nearly six months after the date of inoculation, and that higher concentration of sugar prevailed earlier in the experiment. The fungus, of course, would use sugar in growth, and even after growth some sugar would be used. This leads to another point, that in the inoculated tubes there is probably an increase in the carbon dioxide content of the air of the tubes. This increased CO_2 content, increased sugar, and higher hydrogen ion concentration would suffice to explain the accelerated rate of germination.

Regarding infection, prepared slides were carefully examined. Those inoculated on March 22 were infected by the organism, and some small ones were completely invaded. There were very few completely invaded, however, and the mortality was slight. While

individual embryos and seedlings were not sectioned, it was possible to examine the sections serially by the use of a mechanical stage. Some of the seedlings in those cultures inoculated on March 22 were not infected, and more appeared not to be infected in those inoculated on April 27.

When starch is provided as the organic matter in the culture media, there is no germination unless the fungus is supplied. The explanation of the action of the fungus cannot be ascribed to any internal changes induced by the fungus, since many embryos were noted which were not infected. The external changes induced by the fungus are alone sufficient to induce germination under these conditions.

TABLE IV

Cattleya HYBRID SEEDS INOCULATED AND PLANTED DECEMBER 21, 1922; NOTES ON RA 28 AND RA 30 MADE JANUARY 2, AND ON RA 25 AND RA 31 MADE JANUARY 29, 1923

Culture solution and condition	Culture no.	Percentage killed	Average diameter (μ)	P_H
B+2 per cent sucrose	RA 28	0	207	5.6
B+2 per cent sucrose, inoculated	RA 30	12	225	4.2
B+2 per cent sucrose	RA 25	4	316	5.6
B+2 per cent sucrose, inoculated	RA 31	37	486	4.2

EXPERIMENT 4.—What will be the effect of the fungus on germination when sugar is supplied in place of starch? Experiments, reported previously, demonstrated that germination is possible with sugar, and according to BERNARD and others the sugar would be a substitute for the fungus. One might expect, therefore, that in cultures containing sugar the fungus would be without any accelerative effect, since a substitute is provided. Or one might expect that if the fungus is of value, due to inducing internal changes, the fungus would thereby favorably influence the embryos. There is finally the possibility that the fungus might accelerate or retard germination because of changing the chemical reaction of the medium.

The cultures were inoculated on the same day that the seeds were planted, and the cultures were kept in the laboratory for three days previous to being transferred to the greenhouse. Within the first two weeks the fungus had accelerated the rate of growth, and

by January 21, one month after sowing, those embryos with the fungus were much superior to those without the fungus.

The embryos of RA 31 (inoculated) were dark green and the leaf point was well defined. The embryos in RA 25, however, were light green and the leaf point was not apparent in any of the embryos. The fact to be noted from the data is that the fungus markedly accelerated germination. The favorable influence of the fungus is here explainable by the change in hydrogen ion concentration of the culture medium. The inoculated cultures had a P_H of 4.2, those uninoculated 5.6. The difference in color and greater growth may be attributed entirely to the more favorable hydrogen ion concentration. This fact has been well demonstrated by experiments made in this laboratory but not yet published.

Seedlings from RA 28, RA 30, and RA 31 were prepared for microscopical examination. RA 28 not being inoculated was of course free from infection; RA 30 and RA 31 showed some embryos completely invaded. Some were but slightly invaded, and others were not infected.

EXPERIMENT 5.—This experiment was essentially like experiment 4, but KH_2PO_4 was used in the culture medium and the seeds were of a different hybrid. The experiment was made at the same time as experiment 3. The data are given in table V.

On October 3 additional notes were made. The inoculated sucrose cultures were superior in size to the uninoculated, and likewise were of a deeper green. Due to the crowding of seedlings, growth had been restricted, and the difference apparent on October 3 would undoubtedly have been greater had more favorable growth conditions been provided. In again looking for an explanation of the favorable influence of the fungus on germination in the sucrose cultures, hydrogen ion and reducing sugar determinations were made on May 15. Solution B+2 per cent sucrose not inoculated had a hydrogen ion concentration represented by P_H 5.6, and the amount of reducing sugar in the culture medium was 15.2 mg. In the inoculated culture the hydrogen ion value was P_H 4.0, while the amount of reducing sugar was 141.2 mg. The amount of culture medium used was 8 cc. to each tube, which theoretically (deducting for 10 per cent moisture in the cane sugar) should have contained approximately 144 mg.

sucrose. The facts of significance here are the change of sucrose to hexose sugars by the fungus, and the change in hydrogen ion concentration. From previous experiments not yet published I am inclined to believe that the inversion of cane sugar is of no particular significance, but the shift in the hydrogen ion concentration is of great importance.

Embryos and seedlings taken from these cultures were prepared for examination. In four or five embryos out of nearly forty examined from culture SA 12, infected cells were noted, but most of the embryos were entirely free of any infection. In the inoculated cultures SA 17, SA 18, and SA 23 not a single embryo or seedling

TABLE V

Cattleya HYBRID SEEDS PLANTED MARCH 15, 1923; CULTURE SOLUTION B+2 PER CENT SUCROSE

DATE OF INOCULATION	CULTURE NO.	AVERAGE DIAMETER OF EMBRYOS (μ)				OBSERVATION MADE JUNE 15
		April 12	Culture no.	May 16	Culture no.	
Not inoculated	SA 7	234	SA 9	387	SA 8	Light green; 5 per cent germinated; 5 per cent dead
March 22	SA 12	261	SA 17	621	SA 18	Dark green; 90 per cent germinated
April 27	.....	.....	.....	.....	SA 23	Dark green; 90 per cent germinated

was found to be infected, showing that the action of the fungus is not within the embryo but in the culture medium.

EXPERIMENT 6.—In this experiment another *Cattleya* hybrid was used. The results were like those of experiment 5. There was again a marked increase in growth in the inoculated over the uninoculated culture. The cause of this increase is due entirely to the increase in hydrogen ion concentration. None of the embryos or seedlings when examined in prepared slides showed any infection. Apparently these embryos, as well as those in the preceding experiment, gained immunity as a result of having available a supply of utilizable sugar. Other experiments of similar character were made with like results. The data are given in table VI.

EXPERIMENT 7.—If the action of the fungus is merely on the nutrient solution and without any special value within the embryo,

then a nutrient solution having the same concentration of sugar and the same hydrogen ion concentration as an inoculated culture should accelerate germination to the same degree. That such is the case is apparent from the results of the following experiment. In one series solution B containing approximately 0.05 per cent glucose was used, while the second series contained solution B+0.25 per cent starch.

TABLE VI

Cattleya HYBRID SEEDS INOCULATED AND SOWN FEBRUARY 20; NOTES TAKEN MARCH 20, 1923

Condition of culture solution	Culture no.	Percentage killed	Average diameter (μ)	P_H
Inoculated	DEL I	8	604	4.0
Not inoculated	DE I	5	307	5.6

The cultures of the starch series were inoculated on March 22. The seeds were sown on March 15. The data are recorded in table VII. Here the interesting fact is to be observed that the inoculated starch culture having the same hydrogen ion concentration and approximately the same quantity of sugar made almost identically the same growth in the same period of time as was found in the

TABLE VII

Culture solution	Average diameter of embryo (μ)	P_H of culture medium	Glucose in culture (mg.)
Solution B+0.05 per cent glucose	239	4.0	3.2
Solution B+0.25 per cent starch inoculated	246	4.0	3.7

glucose culture. Certainly the infection of the embryo was of no special value in this case.

EXPERIMENT 8.—In all the preceding experiments the culture medium contained either starch or sugar. The objection might be made that such a medium is not at all comparable with the substrate on which orchids grow. Such an objection may be granted, but the experimental basis for the symbiotic view is based entirely on the use of such media in culture work. In order to answer such criticism, however, an experiment was made in which a mixture of equal parts

of *Osmunda* fiber and *Sphagnum* was used, such as is often employed in the germination of orchid seeds. Thirty-seven gm. of such a mixture was placed in each of eight Erlenmeyer flasks of 500 cc. capacity. To each was then added 110 cc. of solution B, adjusted to a hydrogen ion concentration of P_H 4.6 by the addition of hydrochloric acid. This solution contained neither sugar nor starch. These flasks were then plugged with cotton and sterilized for thirty minutes at 15 pounds pressure. Six of these flasks were then inoculated on May 20 with the orchid fungus and incubated at 26° C. On June 9, by which time the fungus had grown throughout the upper half of the material, two of the flasks were re-sterilized to kill

TABLE VIII

Cattleya HYBRID SEEDS SOWN JUNE 4; NOTES OCTOBER 4, 1923

Treatment	Culture no.	Average diameter (μ)	P_H of solution	Total reducing sugar present (mg.)
Control, no fungus.....	SE 1	495	4.4	18.2
Control, no fungus.....	SE 2	531	4.4	480.0*
Inoculated May 20....	SE 4	531	4.4	40.0
Inoculated May 2.....	SE 6	531	4.4	10.8
Inoculated May 20....	SE 8	378	4.4	40.0

* Contaminated with *Penicillium* sp.

the fungus. There remained two flasks which had not been inoculated and which were the controls. On June 10, seeds of a *Cattleya* hybrid were planted in all the flasks, and these were then placed in the greenhouse. Observations were made on all these flasks on October 4, 1923. The data are given in table VIII.

The hydrogen ion concentration was determined by extracting 5 gm. of the medium with 10 cc. of distilled water. This was then centrifuged and the supernatant liquid used for the determination. Sugar was only roughly determined by extracting the entire mass for 24 hours with 95 per cent alcohol. The whole mass was then thrown on to filter paper and the alcohol drained off. This mixture was then washed with alcohol, and the alcoholic filtrate placed on a water bath. When dry, the residue was redissolved in 100 cc. of distilled water, filtered, cleared by means of neutral lead acetate, and the lead removed by means of sodium sulphate. The filtrate

obtained was then analyzed for reducing sugar. The high hydrogen ion concentration prevailing at all times rendered it unnecessary to examine for sucrose.

In cultures SE 1, 2, and 6 various seedlings were noted having one leaf, and in others the leaf was just appearing. In culture SE 8 only one seedling with a leaf was noted. Cultures SE 3, 5, and 7 became contaminated with a *Penicillium*, but a few seeds near the walls of the flasks which were not covered by the fungus made excellent development, being superior to any of the others.

It is obvious from table IX that the inoculated cultures were no better than those of the control cultures which lacked the fungus. SE 2 was contaminated with *Penicillium* sp., which accounts for the high content of sugar. The growth of the fungus was markedly less in the SE 6, which may account for the less sugar. The fact of greatest significance is that germination occurred in the flask lacking any fungus. If the orchid fungus is essential, we should not expect germination under these conditions.

The objection might be made that the material was sterilized; but certainly the concentration of sugar was low, only 18.2 mg. in about 100 cc. of culture solution. Again, objection might be made on the basis that solution B was added, and that the hydrogen ion concentration was adjusted; but, with the same hydrogen ion concentration, germination has not been obtained on solution B in any agar cultures. The evidence points again to the conclusion that germination of orchid seed is absolutely dependent on soluble organic food obtained from external sources.

EXPERIMENT 9.—This experiment deals with the effect of various fungi on germination of orchid seeds. It has been shown in the preceding experiments that the orchid fungus will induce germination when the seeds are planted on medium containing starch, and likewise that the germination of seeds is accelerated when sugar is supplied. The explanation offered is the change in hydrogen ion concentration in the sucrose cultures, and in the starch cultures the increase in hydrogen ion concentration combined with the formation of sugar. In the light of these facts it may confidently be expected that any fungus which can digest starch, changing it to sugar, and likewise increase the hydrogen ion concentration, should be capable

of inducing germination, provided that the growth of the organism is not great enough to smother the seed, or provided that the fungus is not capable of infecting and killing the embryos.

TABLE IX

EFFECT OF VARIOUS COMMON FUNGI ON GERMINATION OF *Cattleya* HYBRID; SEEDS SOWN APRIL 14; NOTES MADE AUGUST 19, 1923

Series	Fungus	Date of inoculation	Condition
A.....	Phytophthora sp.	6–15–23	All very good; none dead; dark green and healthy looking; decidedly better than checks
B.....	Sclerotinia libertiana	6–15–23	All plants dead and brown
C.....	Oidium lactis	6–15–23	Fungus failed to grow; no better than checks
D.....	Sclerotinium sp.	6–15–23	All plants dead and brown
E.....	Choanophora cucurbitarum	6–15–23	Plants still green and healthy looking; not as good as A but better than checks
F.....	Penicillium camembertii	6–15–23	Plants in four of tubes green and healthy but developing slowly; in fifth tube three well developed fern prothallia present, apparently as contamination of orchid seed; in this tube orchids growing much more rapidly, darker green, and looking slightly better than those in series A
G.....	Corticium vagum	6–15–23	Good development at first but now turning brown; two of tubes have fern prothallia in them and here seeds appear better than in remainder
H.....	Mortierella rhizogena	6–15–23	Seed is practically all smothered by dense growth of fungus; those still uncovered look better than checks
I.....	Cucurbitaria sp.	6–15–23	Seeds still healthy; dark green and developing much better than checks
J.....	Cladosporium citri	6–15–23	Now becoming smothered by fungus; those not covered are of good green color and better than checks; one tube has two fern prothallia
K.....	0–22 (orchid fungus)	7–19–23	Much better than checks; dark green and developing rapidly; about equal to series A
L.....	No fungus	6–15–23	Seed still green but very small; decidedly inferior to those in series A, E, F, I, and J

Solution B+0.5 per cent soluble starch was used and 10 cc. was added to each tube. The tubes were then plugged, sterilized, and sloped. Some days after planting, the tubes were inoculated, and notes taken, as indicated in table IX. It may be noted that in many cases where the seeds were not covered or killed by the fungus, the embryos were more advanced than in the control cultures. The

cultures inoculated with *Phytophthora* sp. were especially favorable to growth of the embryos. Here we have suggestive evidence of the favorable action of various fungi. Unfortunately, the rate of growth of some of the fungi was too great, and most of the seeds were covered by the developing mycelium.

Further observations were made on these cultures on October 27, and the data are given in table X. At this time hydrogen ion determinations and reducing sugar determinations were also made.

TABLE X

Cattleya HYBRID SEEDS PLANTED APRIL 14; NOTES TAKEN OCTOBER 27, 1923

Series	Fungus used	Average width of embryos	Percentage with leaves	P_H of solution	Reducing sugar present	Notes
A.....	Phytophthora sp.	432	11	5.0	.....	.....
A.....	Phytophthora sp.	369	5	5.1	13.5	Starch in tube entirely digested
A.....	Phytophthora sp.	423	10	5.0	22.8	Starch in tube entirely digested
K.....	Orchid fungus	468	10	4.6	.....	Starch in tube entirely digested
K.....	Orchid fungus	477	16	4.5	16.9	Starch in tube entirely digested
K.....	Orchid fungus	468	11	4.5	11.1	Starch in tube entirely digested
E.....	Choanophora cucurbitarum	288	4*	5.4	0.0	Starch in tube entirely digested
G.....	Corticium vagum	261	0†	6.4	1.8	Starch in tube entirely digested
L.....	No fungus	261	0	6.4	1.8	Starch abundant in tube
L.....	No fungus	243	0	6.4	1.4	Starch abundant in tube
L.....	No fungus	225	0	6.4	.....	Starch abundant in tube

* Seeds germinated only where not covered by fungus.

† Seeds smothered by fungus; in one tube two seedlings were produced somewhat later.

Table X shows that the *Phytophthora* sp. (isolated from Easter lily) is about as effective as the orchid fungus in inducing germination. There is a slight difference in the size of the embryos, but this may be attributed to the lower hydrogen ion concentration. With *Choanophora* a few seedlings were produced. These were just above the mycelial mat. This organism grew more vigorously than either the orchid fungus or *Phytophthora*, which probably accounts for the complete absence of sugar in the culture medium, although the starch had been entirely digested. With *Corticium vagum* the starch was entirely digested. little sugar was present, but the hydrogen ion

concentration (P_H 6.4) was unfavorable to growth. The embryos likewise were enmeshed within the ramifying hyphae. The fungi that induced germination produced comparable changes in the culture medium, namely, increase in the hydrogen ion concentration and digestion of starch with the production of sugar. The concentration of sugar in series K at the conclusion of the experiment (8 cc. remaining of the original 10 cc.) ranged from 0.138 to nearly 0.21 per cent, while in the *Phytophthora* series the concentration ranged from 0.168 to 0.285 per cent sugar. In cultures with *Penicillium camembertii*, and also in the cultures with *Mortierrela rhizogena* a few seedlings were produced near the upper portion of the slope and above the fungus mat. Most of the embryos were either retarded or

TABLE XI

Cattleya HYBRID SEEDS PLANTED FEBRUARY; NOTES MADE JUNE, 1923

Culture conditions	Culture no.	Average diameter (μ)
Solution B+¼ starch, not inoculated	SC 2	225
Solution B+¼ starch, inoculated seed on surface of medium	SC 32	432
Solution B+¼ starch, inoculated but seed adhering to wall of tube and not in contact with agar	SC 32	198

killed when covered by the fungus. Such data permit of the conclusion that the external changes only are important.

Prepared slides were made of a large number of seedlings and embryos taken from all of the series included in table XI. These preparations were carefully made and thoroughly examined, not only with the usual dry objectives, but also at magnifications of 1200. In no case was any embryo or seedling infected. The strikingly beneficial effect of *Phytophthora* and the orchid fungus, therefore, could not have been due to any internal action of the fungus. I did not expect to find infection with *Phytophthora* sp., but I had expected to find it with the orchid fungus. These cultures of series K were inoculated three months after the seeds were sown, however, and apparently the embryos had become entirely immune to the fungus.

EXPERIMENT 10.—BURGEFF, in describing one of his experiments with the fungus, stated that the embryos adhering to the inner

surface of the tube out of contact with the culture medium germinated. I suggested that possibly the embryos were in contact with droplets of the agar medium adhering to the surface, and that the products of digestion or excretion were adequate to account for growth. There was also the possibility that, in examining the tube, seedlings accidentally became detached from the slope and were flipped to the side of the tube. If such embryos on the side of the tube could germinate when out of contact with the culture medium, then we would have rather strong evidence in favor of the fungus hypothesis.

That embryos adhering to the tube wall out of contact with agar do not germinate, and are no better than the embryos on starch media uninoculated, has been noted many times in the course of my experiments. In at least eight different inoculated cultures with solution B+$\frac{1}{4}$ starch, seeds have been noted adhering to the wall of the tube, and no stimulating effect of the fungus was noted. In table XI, data are given on the growth of seeds on the walls and on the agar surface of an inoculated starch medium. For comparison, there are given the growth measurements for the uninoculated starch culture. The embryos adhering to the wall were all infected, so that lack of infection was not responsible for any failure to germinate. They were likewise of the usual green color, but not having available sugar and possibly also organic acids produced by the fungus, germination was not possible.

EXPERIMENT 11.—That the effect of the fungus on orchid seed germination is external and not internal is further shown by the following experiment. Six series of cultures were prepared with solution B and various concentrations of starch. The inoculated series had starch contents of $\frac{1}{4}$, $\frac{1}{2}$, 1, and 2 per cent respectively. The control cultures consisted of one series with $\frac{1}{4}$ per cent starch and a second with 2 per cent starch. The treatment of these two series was identical with the preceding, except that they were not inoculated. All of these cultures were planted with the same seeds and maintained under the same conditions. On January 10 measurements were made of the embryos, the figures given under average width being again the average of fifty measurements. Hydrogen ion concentration and reducing sugar were also determined. The data

are given in table XII. It may be noted that, in the two control cultures with $\frac{1}{4}$ and 2 per cent starch, not inoculated, the average size of the embryos is only 124 μ. In the inoculated cultures there is an increase in growth. The higher the concentration of starch the greater the sugar content, and therefore the greater the growth. It may be noted also that the hydrogen ion concentration of the inoculated cultures was increased in all cases to P_H 4.7 by the fungus.

These data give emphasis again to the external changes as the important factor in germination under pure culture conditions. Certainly if the effect of the fungus were within the embryo, we should not expect differences of the character observed.

TABLE XII

Cattleya HYBRID SEEDS PLANTED NOVEMBER 10, 1923; NOTES MADE JANUARY 10, 1924

Culture solution	Inoculated	Culture no.	Average width of embryos (μ)	Maximum width of embryos (μ)	P_H of culture medium	Glucose per culture tube on January 10 (mg.)
Solution B+$\frac{1}{4}$ per cent starch..	December 11	SL 2	211	291	4.7	5.9
Solution B+$\frac{1}{4}$ per cent starch..	Not inoculated	SM 7	124	144	5.5	0.0
Solution B+$\frac{1}{2}$ per cent starch..	December 11	SM 1	268	384	4.7	28.7
Solution B+1 per cent starch..	December 11	SN 1	311	480	4.7	63.8
Solution B+2 per cent starch..	December 11	SO 2	393	576	4.7	121.8
Solution B+2 per cent starch..	Not inoculated	SO 7	124	144	5.5	0.0

EXPERIMENT 12.—Some of the previous experiments indicated that there is a relation between the physiological state of the embryos and the degree of infection. This was shown in experiment 1, as well as in other experiments. Some of the data suggested that the longer the interval of time between the date of sowing and the date of inoculation, the less would be the destructive action of the fungus.

In order to determine specifically whether or not the interval of time between the date of sowing and the date of inoculation is a factor to be considered, the following series of experiments was started. Eighty tubes were prepared with solution B, using KH_2PO_4 instead of K_2HPO_4, and $\frac{1}{4}$ per cent starch. Beginning on October 24, ten tubes were planted with seeds of a *Cattleya* hybrid. At in-

tervals of every two days until November 9, ten more tubes were sown with the same seeds, so that the last seeds were sown sixteen days later than the first. Then on November 9 five tubes of each series were inoculated with the same culture of the orchid fungus. By this procedure some cultures were inoculated sixteen days after the seeds were sown, others fourteen days later, others twelve days after sowing, and so on, until one lot of cultures was inoculated on the same day that the seeds were planted.

At intervals of time the cultures were examined, and finally on April 11 notes were taken on these cultures. In the uninoculated cultures the embryos were in the small spherule stage, being approximately 220 μ in diameter. They were green and still living. In the inoculated cultures the results were all alike. Of approximately 200 seeds planted in each tube, only one or two germinated. The remainder were white or brownish, varying in diameter from 180 to 450 μ. The smaller embryos were heavily infected, and masses of hyphae radiated from the embryos. The larger embryos were also heavily infected and the cells appeared to be disorganized, showing the pathogenic character of the fungus.

That the activity or the capacity of the fungus was not altered by being kept under pure culture conditions is evident from the following experiment. Ten of the original control cultures (uninoculated) were inoculated with a subculture of the same organism on April 11, 1924. Others of these control cultures were inoculated with the fungus from *Epipactis* and *Cypripedium*. On May 10, most of the embryos were just producing the leaf point and only a few were killed. By May 22 most of the seeds had germinated. The embryos in cultures of the previous experiment inoculated sixteen days after planting were practically all killed, but the embryos of this lot inoculated six months later with the identical fungus, or inoculated with the fungus from *Epipactis* or *Cypripedium*, were capable of germination, only a few of them being killed. This resistance on the part of these embryos is owing to physiological or cell wall changes due to prolonged culture, or else the embryos become resistant because of a higher starch content of the medium, and consequently on digestion of starch by the fungus a higher sugar concentration.

When the tubes were prepared the concentration of starch was 0.25 per cent, but as a result of evaporation the uninoculated tubes had a starch concentration of approximately 0.5 per cent on April 11. Previous experiments with media containing sugar or high starch content, which when inoculated is equivalent to high sugar content, showed that the embryos gain immunity by being supplied with a relatively high concentration of sugar. This aspect of the subject is being investigated at the present time.

EXPERIMENT 13.—One of the strongest arguments advanced in favor of the symbiotic view is that the fungus from one genus of orchids may be ineffective in causing germination of seeds of orchids of other genera. While the fungus from *Cattleya* is effective for the seeds of *Cypripedium*, *Cymbidium*, and *Epipactis*, it is claimed to be without effect on the germination of seeds of *Odontoglossum*. Likewise the fungus of *Odontoglossum* does not bring about germination of *Cattleya* seeds. Lacking plants of *Odontoglossum*, I have not as yet been able to obtain this fungus, but the effect of fungi isolated from *Cattleya*, *Epipactis*, and *Cypripedium* on seeds of *Odontoglossum* was determined.

For determining the effect of these fungi, five tubes of solution B+$\frac{1}{4}$ starch were sown with seed of an *Odontoglossum* hybrid, and inoculated with the fungus from *Cattleya*. This was repeated with the fungus from *Epipactis* and *Cypripedium*. Another similar series was provided, using $\frac{1}{2}$ per cent starch in place of $\frac{1}{4}$ per cent. With these fungi there was no germination. Upon examination it was found that every seed had been killed. Most of the seeds were completely invaded, and masses of hyphae were present. The failure of the fungi isolated from *Cattleya*, *Cypripedium*, and *Epipactis* to produce germination is due, not to failure to infect the seeds, but to the extremely pathogenic character of the fungus for the seeds of *Odontoglossum*. Seeds of *Odontoglossum* sown on solution B+2 per cent sucrose adjusted to P_H 4.5 germinated and made excellent growth.

Summary of experiments

1. A fungus resembling morphologically that described by BERNARD as *Rhizoctonia repens* was isolated from *Cattleya*, *Cypripedium*, and *Epipactis*.

2. This fungus was capable of inducing seeds of *Cattleya* to germinate on a medium containing starch.

3. This fungus accelerates the growth of orchid seeds in solution B containing sucrose.

4. The fungus may permit of complete germination or may kill every seed in the culture. One of the factors which controls the degree of infection is the concentration of starch used. This means, of course, that ultimately it is the concentration of sugar.

5. In the culture medium containing starch in which germination occurs when the fungus is present, the following changes occur. The starch finally is completely digested, and sugar is produced. The hydrogen ion concentration is changed from a value which is unfavorable for growth, to one which is favorable. These external changes are sufficient to bring about germination

6. Germination may be effected by the fungus, even though no seeds are infected.

7. The fungus hastens germination of seeds in solution B+2 per cent sucrose. The acceleration is due to the increase in hydrogen ion concentration, and not to any internal action, for the embryos are not infected.

8. Germination was obtained without the fungus on a peat and sphagnum mixture with solution B adjusted to P_H 4.6, and the germination was just as rapid as when the fungus was supplied. The fungus is without any value here.

9. The growth of the embryos with solution B+0.05 per cent glucose, with a hydrogen ion concentration P_H 4.6, is about the same as with solution B+$\frac{1}{4}$ per cent starch inoculated with the fungus. The sugar content and hydrogen ion concentration of the latter were practically the same as those of the glucose culture.

10. *Phytophthora* sp. is about as favorable to germination as the orchid fungus. The chemical changes induced in the culture medium by the former are nearly the same as those produced by the orchid fungus. Germination was effected by other fungi.

11. In an experiment in which solution B was used with concentrations of starch varying from $\frac{1}{4}$ to 2 per cent, with the fungus, the growth increased with the concentration of starch. With $\frac{1}{4}$ per cent starch, germination was not attained, even after six months. For

each increase in starch content there resulted, because of digestion by the fungus, a progressive increase of sugar. The hydrogen ion content was the same in all cases.

12. In determining the effect of the fungi isolated from *Cattleya*, *Epipactis*, and *Cypripedium* on seeds of *Odontoglossum*, it was found that these fungi are extremely pathogenic, and practically every seed was killed. Without the fungus the seeds turned green and were still living after six months; with sugar supplied, the seeds germinated.

Discussion

The symbiotic theory rests very largely upon two facts. The first is that the roots of orchids are generally infected by a fungus, which fungus is generally believed to be of some value to the orchid. At present, however, it must be granted that no evidence has yet been presented which indicates any favorable effect of this infection on the orchid plant. The presence of the fungus in the roots of orchids is not evidence that the fungus is essential. The association of fungus and root may be merely incidental and not of any significance. CONSTANTIN and MAGROU go so far as to state that the relationship is quite intimate, for they practically state that it is no more permissible to consider an orchid, which lacks the fungus, as an orchid, than it is permissible to consider the individual plant components of a lichen as a lichen. The fact remains, however, that no proof has ever been presented to show any beneficial effect of the fungus, unless we accept the conclusions of KUSANO (**9**), who stated that *Gastrodea elata*, a rather unusual Japanese orchid, will not flower unless infected by *Armillaria mellea*. Incidentally this organism is quite distinct from the real orchid endophyte

The proponents of the symbiotic view state that the experiments of BERNARD and BURGEFF prove that the orchid fungus is essential for germination. In my first paper I stated that under the conditions of pure culture, as followed by BERNARD and BURGEFF, the fungus was essential, but I suggested that the fungus was of value in these cases only because it changed starch to sugar or increased the quantity of other soluble organic food as a result of digestion processes and by excretion of favorable substances. The experiments reported in this paper show that the external changes induced by

the fungus are sufficient to explain germination when the fungus is supplied. These changes induced by the fungus are digestion of starch, formation of sugar, and the production of a favorable hydrogen ion concentration. These changes are in themselves adequate for inducing germination. In addition to these facts, however, evidence is presented showing that even on a starch medium, infection of the seedlings did not always occur, although the seeds germinated. Germination on a starch-containing medium was obtained also with fungi not related to the orchid fungus. To be sure this fungus was slightly superior, but the more favorable action of this fungus could be explained on the basis of more desirable changes induced in the culture medium.

BERNARD emphasized the fact that a delicate balance had to be maintained between the fungus and embryo. He states: "The germination by inoculation is not without certain difficulties. . . . . For the majority of seeds, the association with the fungus that I have placed in their presence has been merely passive and without effect, or impossible or rapidly injurious to the embryos."

CONSTANTIN and MAGROU have criticized me for quoting BERNARD in this manner. This quotation was made to lend emphasis to the idea that the fungus was at times a real pathogen. I stated in my first paper as follows: "It is possible that the fungus instead of being an aid in normal germination, is a factor in the death of the embryos and consequently in the failure of germination." That such is the case must be apparent from data supplied in experiment 12, where less than 1 per cent of the seeds were able to germinate after a period of six months. In the control cultures not inoculated the seeds were still all alive.

One of the arguments that might be offered in favor of the symbiotic conception is from the experiments of BERNARD on the difference in "activity" of the fungus when isolated from different species of orchid, and also the so-called loss in activity when the fungus is grown under pure culture conditions. If the interpretation put upon these experiments by BERNARD were accepted, it would be necessary to conclude that symbiosis plays a rôle in orchid seed germination, but I am convinced that this interpretation is not correct. Before considering in detail one of these experiments, it is necessary to

define what BERNARD means by activity. He states: "I have considered that fungus the most active which, all other conditions being equal, causes the most rapid germination, the greatest number of seedlings, or the best developed seedlings in a given time." In this particular experiment BERNARD determined the influence of the duration of pure culture conditions on the activity of the fungus. The initial isolation of the fungus was made December 1905, and, of course, repeatedly transferred during the subsequent three years, when finally the activity of the fungus was compared in its influence on germination with the activity of subcultures of this same fungus. The different subcultures during the three years, however, had

TABLE XIII

Culture designation	Date of isolation	Origin	Subsequent treatment	Pure culture
Mycelium C..	December, 1905	.....	.....	3 years
Mycelium C_1..	June 6, 1905	C	March 1 to June 6, 1906, in Laelio seedlings (67 days)	19 months
Mycelium C_2..	October 26, 1906	C_1	June 15 to October 26, 1906, in Laelio seedlings	14 months
Mycelium C_3..	May 3, 1907	C_2	November 14, 1906, to May 3, 1907, in Cattleya seedling	8 months
Mycelium C_4..	November 14, 1907	C_3	July 1 to November 14, 1907, in Cypripedium seedling	1.5 months

each a different history. The fungus kept under pure culture conditions throughout the period was labelled C, but C_1 had the following history. It was used for inoculating seedlings of *Laelio* on March 1, 1905, and reisolated on June 6, 1905. Thereafter it was maintained under pure culture conditions. The history of the other subcultures is indicated in table XIII. The results were striking. In the tube inoculated with culture C, the seeds did not exhibit any development whatsoever; with culture C_1 only a single seedling was produced, with but a very small percentage of seeds which showed any appreciable development; with C_3 approximately 5 per cent of the seeds produced seedlings; while with C_4 a high percentage of seedlings was produced. The data indicated that the shorter the time between the date of isolation and the date when the fungus is used for inoculation, the more effective is the fungus in inducing germination.

Assuming that the orchid cultures were all maintained under

identical conditions, one would have to conclude that the difference in the life histories of the fungus is a factor in its ability to induce germination. This may be accepted, but the explanation is not a loss of activity in the sense that the seeds are not injured, but the failure of germination is due to the fact that the fungus has behaved as a strong pathogen, and with cultures C and C_1 most of the seeds were killed by the fungus. In other words, the most active fungus is the one which is the weakest pathogen.

In the course of my various experiments with the fungus I have had germination varying from 0 to 100 per cent. I have not as yet had the opportunity to determine precisely what conditions make for a low or a high percentage of germination. In general, however, the results indicate that the higher the starch content of the culture medium, the higher will be the sugar content of the medium, and consequently the lower the mortality. The composition of the nutrient solution, the length of the interval between the date of sowing and the date of inoculation, and the kind of seeds used are all factors to be considered. Some preliminary experiments indicate also that the composition of the medium on which the fungus has grown is a factor in the virulence of the organism.

Another argument advanced by RAMSBOTTOM in favor of the symbiotic conception is that the fungus of *Odontoglossum* is without any effect on *Cattleya*, and likewise the *Cattleya* fungus is without any effect on *Odontoglossum*. I have tested the fungi isolated from *Cattleya*, *Epipactis*, and *Cypripedium* on *Odontoglossum* seed, and in each case the seeds were killed. The significant fact is that the fungi from *Cattleya*, *Cypripedium*, and *Epipactis* all killed seeds of *Odontoglossum*. This being so, it is possible that one of the difficulties in the germination of *Odontoglossum* seeds is that the fungus from *Cattleya* is present in the greenhouse, and is particularly destructive to the embryos of *Odontoglossum*.

Other evidence that might be cited from the work of BERNARD in favor of the symbiotic view of orchid seed germination is his experiments with *Bletilla hyacintha*. He found it possible to germinate seeds of this orchid without the use of any fungus, but the seedlings produced were somewhat attenuated. With the fungus the seedling formed a protocorm and was more compact in structure.

BERNARD maintained that the fungus, through internal action, induced chemical changes resulting in a high concentration of sap. The high concentration of sap in turn caused the difference in the type of seedling. BERNARD'S evidence that the fungus causes an increase in concentration of the cell sap was that he grew the fungus in a nutrient solution containing salep and sucrose, and found that it produced an increase in concentration of the nutrient solution, this increase being due largely to the inversion of sucrose and digestion of starch. Here was a clue to a rational explanation of the function of the fungus in his experiments, but, from the fact that the fungus brings about an increase in concentration of the nutrient solution, he assumed that the same may take place in the embryo.

In interpreting the differences in the structure of *Bletilla* seedlings with and without the fungus, one must take into consideration a number of facts. In the first place, any plant growing in a small inclosed tube or flask which is tightly stopped with cotton will be restricted in growth due to a decreased rate of photosynthesis. In another paper (**8**) I presented evidence to show that the cotton stopper impeded the diffusion of carbon dioxide into the vessel. MOLLIARD (**10**) demonstrated that radish plants growing in flasks under pure culture conditions produced enlarged roots only when a relatively high concentration of sugar was provided. BERNARD explained the formation of storage roots on the basis of concentration, but the logical explanation is that the storage root is formed only when sufficient food is present.

The compact type of seedling formed by *Bletilla* when the fungus is present is explainable then on the fact that the fungus changes insoluble foods in the salep to soluble foods, and that as a result of respiration of the fungus the carbon dioxide content of the tube is augmented over that in tubes without the fungus.

In considering finally the evidence for the necessity of the fungus for germination, and, as CONSTANTIN and MAGROU state, the necessity of the fungus for the normal development of the plant, it must be granted that the only evidence is the almost constant association of fungus and the orchid plant. That the fungus is necessary for growth of the orchid is not true. In my second paper I described an experiment in which seed of *Cattleya* were germinated without the

fungus, but with sugar. When the embryos were about 0.6 mm. in diameter, they were transplanted to a nutrient solution entirely lacking in any organic food. These seedlings were transplanted several times. The largest seedling now, after three years of growth, has five large leaves 8–15 cm. in length, and well formed pseudo bulbs. The plant, except for the first two months, has been supplied merely with solution B, and has been grown entirely within a flask. Any orchid grower would pronounce it a very good three-year-old plant.

The explanation of the failure of orchid seeds to germinate when provided with all the conditions that permit of the germination of most seeds, is to be found in the organic food relations. The seeds of orchids are lacking in food reserves. There is no endosperm or cotyledon, but the seed is a simple undifferentiated embryo. I have made various analyses of seeds of *Cattleya* for sugar, starch, and fat (ether extract). No starch was present; the total sugar amounted to 1.2 per cent; while the fat content was equal to 32 per cent of the dry weight. Growth of the embryo will continue for a time at the expense of the reserve food, but ceases sooner or later. If the embryos are then supplied with sugar, growth will continue and germination occur. As presented in my second paper, this suggests that orchid embryos are dependent on an outside source of organic food for continued development. This must signify that even in those embryos that are provided with chlorophyll, photosynthesis is lacking. It would seem that photosynthetic activity in the orchid is delayed, due to the lack of some internal factor, as reported by BRIGGS (**2, 3**) for other seeds. If the embryos are carried over this critical period, then they are thereafter self-sustaining.

The significant fact has been noted that the seeds of terrestrial orchids may germinate in nature under conditions where chlorophyll is entirely lacking. These embryos are purely saprophytic. The conclusion seems to be warranted that under natural conditions the orchid embryos are dependent for continued development on an appropriate supply of organic food, which must be absorbed from the material on which the seeds are germinating. Under natural conditions this food is made available to the orchid embryo by the digestion of organic matter, which transforms the insoluble sub-

stances to soluble products. Some of these substances are absorbed by the embryos and are used in the metabolic processes. Under natural conditions the orchid fungus may function in these digestive processes, but it would be pure assumption to conclude that no other microorganisms are involved in this transformation. Sugars are undoubtedly formed, although the concentration would be low. It would seem that other substances are more effective. Additional discussion of this aspect of the problem may well await further investigations on the photosynthetic and food relationships of orchid embryos.

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TROPICAL LIGHT WEIGHT WOODS

KARL C. HYDE

(WITH PLATES XXVI–XXVIII)

Light weight woods have come into prominence in the last decade, as the source of material for the manufacture of "buoyant and insulating products." The extent (**15**) to which the wood of *Ochroma* was utilized by the allied nations during the recent war, in the production of lifeboats, refrigerators, floats, and in the construction of parts for aeroplanes is well known. CARPENTER (**4**) studied the wood of *O. lagopus*, and has briefly set forth its anatomical and physical characteristics. He has correlated these characteristics with the efficiency of the products, by showing that the insulating properties of this wood are much higher than those of any other substance in commercial use, this being due to its peculiar histological structure. The properties of its high insulation against heat, its light weight, and proportionately great structural strength, are no doubt its most valuable qualities (**1**). The utilization of these woods for lining refrigerator cars, for cold storage plants, and for iceboxes has become very general during the past four or five years. This has resulted in decreasing considerably the losses formerly experienced in the marketing of perishable products, and has consequently increased the efficiency of the carriers of foodstuffs throughout the world.

As has been pointed out by ROWLEE (**16**), light weight woods fall naturally into two categories: (1) those in which the elements are arranged in a relatively homogeneous mass; and (2) those in which soft parenchymatous masses alternate with more or less continuous bands of thick walled elements. The first are homogeneous, the second laminated woods. This latter type of wood occurs frequently in the tropics, some species presenting a more laminated structure than others. VOLKERT (**20**), in a histological study of *Jacaratia spinosa* (Caricaceae), found that the only lignified elements in the stem are the vessels; that the wood resembles the tissue of a turnip; and upon drying loses its form, and shrinks to a small fraction of its

original volume. The nearest approach to a laminated type of wood in northern latitudes occurs in the Juglandaceae, where very thin tangential bands of parenchyma occur in the annual ring. These are especially prominent in *Hicoria*.

Geographical distribution

Trees producing light weight wood appear to be confined to certain geographical areas, and in general their distribution is limited to the zone shown on SCHIMPER'S (**17**) map as the "luxuriant tropical rain forest." With the exception of *Adansonia*, which includes the "baobab" tree of Africa, the trees are confined to the wet, hot regions of Central and South America.

In Central America the trees are rarely found in the dry regions of the south coast, northwest of Panama, a region much drier than that along the north coast. In Central Panama the conditions are somewhat different. South and east of the Canal Zone, the humid region is not confined to either coast, but involves the whole breadth of land areas, from coast to coast. The rainfall and humidity gradually become greater on the south and west coasts, whereas the north and east coasts become relatively dry as one approaches Venezuela.

On the west coast of Colombia the humid region continues, especially along the foot of the Andes, as far south as southern Ecuador. This is indicated by the large number of species common to the north coast of Central America, and to the west coast of Colombia and Ecuador. It is in this continuously humid, hot belt, centering in Panama, that these trees reach their maximum both in size and numbers. Here are found species of the following genera that produce light weight woods: *Ochroma*, *Heliocarpus*, *Pachira*, *Apeiba*, *Cordia*, *Cavanillesia*, and *Wercklea*. There are no doubt others that will be discovered with further exploration. The majority of trees producing light weight wood belong to the closely related families Tiliaceae (*Heliocarpus*, *Apeiba*), Malvaceae (*Wercklea*), and Bombacaceae (*Pachira*, *Ochroma*, *Cavanillesia*). To the Boraginaceae, a somewhat unrelated family, belongs *Cordia*.

Material and methods

Because of the increased economic importance of light weight woods and of the almost complete lack of literature concerning them,

the writer undertook a detailed histological study of representatives of all the preceding genera, with the exception of *Ochroma*. For the structural study of laminated wood, four species were used which differ markedly in the degree of lamination, as well as in other histological characters. They are *Heliocarpus popayanensis*, *H. appendiculatus*, *Apeiba aspera*, and *Cordia heterophylla*. For the study of homogeneous light weight wood three species were used, namely *Pachira barrigon*, *Cavanillesia platanifolia*, and *Wercklea insignis*.

The method employed in sectioning the wood, in general was as follows. The areas for treatment were carefully selected to avoid knots, season checks, or other irregularities, and blocks approximately 6 mm. square were cut. Care was exercised to expose perfect transverse, tangential, and radial aspects on the surface of the block (3). The blocks were given a thorough boiling to drive out the air, but in doing this it was necessary to be very careful, as a prolonged boiling caused the tissue to crumble. This process did not remove all the air, and it was necessary to use the air pump for this process. The blocks were then treated with a 50 per cent solution of hydrofluoric acid for four to eight days, depending upon the hardness of the wood. After washing out the acid, the blocks were usually imbedded in celloidin, according to the method given by PLOWMAN (11). In some cases it was found that by softening the blocks for two weeks in a mixture of 50 per cent alcohol and glycerine, after removing the acid, satisfactory sections could be cut on the sliding microtome without previous infiltration. For staining the sections, Delafield's haematoxylin in combination with safranin gave the best results. For examining the sculpture of the walls, unstained sections mounted in glycerine jelly were very satisfactory. For studying the individual elements, macerated material mounted in glycerine jelly was used to advantage. To make the elements more easily studied, they were stained in a saturated solution of nigrosin in picric acid, according to the method given by LEE (8). This helped appreciably in outlining them with the camera lucida.

Heliocarpus popayanensis

LOCALITY AND HABIT OF GROWTH

Heliocarpus belongs to the Tiliaceae. It is exclusively a genus of the American tropics, being found in southern Mexico and extending

from this northern limit to Chili. The number of species in the genus is questionable, but according to ROSE (**14**) is between fifteen and twenty. *Heliocarpus popayanensis* was first described in 1821. The type specimen was collected near Popayan (**6**), United States of Colombia. The specimen from which the wood for this study was taken corresponds closely to the type, and was collected between Huigra and Bucay, Ecuador, July 6, 1921, at an altitude of approximately 1000 m.[1] The plant has been reported from Mexico, Ibague, Island of Tobago, Panama, Santa Marta, and Bolivia. The species is sometimes confused with *H. americana*, but differs from it in being a much larger tree, and in having larger and more pronounced trilobed leaves. The tree is called Balsa Menudito in the Magdalena Valley. The natives of Ecuador call it Balsa Bobo, or Fool's Balsa, because of its superficial resemblance to *Ochroma*, the wood of which is known by the Spanish name Balsa throughout Central and South America.

The tree from which this sample was taken was 25 cm. in diameter, and a short distance above the ground divided into several branches. In outward appearance it resembled the American smoke-tree, *Rhus cotinoides* Nutt. It had the general appearance of a plant that was growing out of, or at, the extreme limits of its range. The tree, according to the collector, occurs quite commonly throughout Ecuador. It is closely related to the basswoods of North America.

GROSS ANATOMY

The wood of this tree belongs to the category of a light weight laminated wood. The laminations are very pronounced near the pith, but near the periphery they are scarcely noticeable until sections of the wood are stained. The light weight of the wood is its most striking characteristic. This lightness is due to the thinness and the unlignified character of the walls of the bulk of the elements composing the wood, together with the comparatively large, air-filled lumina. The wood is white with a tendency toward flesh color. There is no perceptible difference in the color of the sap wood and that of the heart wood, but the latter is softer, lighter, less lignified, and less fibrous than the former. The wood is odorless and tasteless. Near the periphery it weighs 19 pounds per cubic feet when oven-

[1] All species discussed in this paper were collected by Professor W. W. ROWLEE, unless otherwise stated.

dried to constant weight at a temperature of 100° C. It has a specific gravity of 0.30. Near the pith it weighs as low as 12 pounds per cubic foot. "Ripple marks" are readily seen with the unaided eye on the surface of the longitudinal cuts. These are more prominent on the tangential than on the radial surface. The height of the markings is fairly constant, and averages 0.38 mm. RECORD (13) has called attention to the presence of "ripple marks" in several other species of the Tiliaceae.

MINUTE ANATOMY

VESSELS.—The vessels as they appear in a transverse section are irregularly distributed throughout the wood (fig. 1). They are more numerous in the lignified than in the unlignified areas. They are mostly round or oval in outline; some of them, however, as a result of crowding in groups, appear to be subdivided radially. The average obtained from fifty measurements of the tangential diameters of the pores was 150 μ, while of the radial diameter, the average of a like number of measurements gave 186 μ. The thickness of the walls varies from 6 to 11 μ, but the average is about 8 μ. The vessels lack tyloses, but a great many of their lumina are partially clogged with mucilage. This occurs in large masses which take a dense haematoxylin stain. The length of the vessel segments varies considerably, fifty measurements giving a maximum of 760 mm., a minimum of 350 mm., with an average of 462 mm.

The walls of the vessels contain many bordered pits. These are arranged alternately, when the vessel wall is contiguous to water conducting elements, so that as a result the whole vessel is covered with diagonal rows of these pits. This alternation of pits is accompanied by crowding to such an extent that the pits, because of their mutual contact, become hexagonal. Bordered pits, in vessels that adjoin medullary ray cells, occur in isolated groups that are banded into vertical and horizontal rows. These groups usually contain nine pits in three rows. The pits on vessels that lie adjacent to water conducting elements are bordered on both sides of the middle lamella, but where the vessel is in contact with medullary ray cells, or wood parenchyma cells having simple pits, the pits in common with the two elements are half-bordered. The diameter of the pits in the vessel wall averages 10 μ. The mouth of the pit is elliptical and is 6 μ

wide. Occasionally, where the vessel joins with ray cells, there is a tendency for the pits to lose their borders. These pits sometimes become greatly elongated, and often anastomose, giving the vessel wall a scalariform appearance. SOLEREDER (**18**) found in the related genera *Echinocarpus*, *Elaeocarpus*, and *Sloanea* that the pits become simple and elongate in a similar manner. The cross walls of the vessel segments vary from transverse to strongly oblique. The terminal apertures of the vessels are always of the porous type.

WOOD FIBERS.—In transverse section the lignified wood fibers appear in wavy bands more or less concentric with the rings of growth (fig. 1). Several of these bands may occur within the limits of a single growth ring. Within these zones the fibers are arranged in uniform radial rows. Alternating with these lignified zones are similar bands of unlignified fibers, giving the whole a decidedly laminated appearance. The lignified areas make up approximately 60 per cent of the wood, while the remaining 40 per cent is composed of the parenchymatous areas. The wood fibers vary in length from 0.6 to 1.4 mm., their average length being 0.97 mm. The width of the medium portion is 32 μ and the thickness of the wall 7 μ. The width of the lumina varies from 12 to 38 μ, with an average of 25 μ.

In a longitudinal section the typical characters of the fibers show to advantage. They appear as slender, elongate elements, spindle-shaped and sharp pointed. They run parallel to one another and exhibit very little, if any, interweaving. They are arranged in a distinct storied fashion.

The study of macerated material often shows the wood fibers with sawlike edges, due to the fact that these fibers are developed adjacent to the medullary rays, the cells of which have left their imprint on the fibers (fig. 18). A few fibers were found with forked ends; others were decidedly flattened.

In the walls of the fibers the pits are slitlike and extremely elongated (figs. 18, 26). They extend beyond the oval outline of the pit membrane, which is exceedingly narrow. In some fibers the outlines of the pit membranes do not coincide, but appear at right angles to one another, giving rise to a cross-sword appearance. The pits usually appear to be scattered, without any apparent regularity. Sometimes, however, they appear in isolated groups or even in longi-

tudinal rows. RECORD and others have called attention to the fact that these localized pit areas, upon the fibers, in some woods tend to make the ripple marks more distinct. The pits usually appear at an oblique angle to the long axis of the fiber (fig. 18).

MEDULLARY RAY.—The medullary rays are very pronounced and of two types, uniseriate and multiseriate, the latter being outnumbered by the former approximately two to one (fig. 2). The multiseriate rays are often six or seven rows of cells wide tangentially. In a transverse section the rays are seen to run continuously through the lignified and unlignified bands, and appear to widen tangentially within the lignified zone (fig. 1). In some areas near the pith, the rays appear to merge into the parenchymatous bands of tissue to such an extent that they are very difficult to delineate.

In tangential section the rays are from a few to many cells high (fig. 2). The multiseriate cells are often as high as seventy cells. That the rays are heterogeneous is clearly brought out by a study of the radial section. In this aspect the rays present a muriform appearance, consisting of many rows of cells. Some of these cells have their long axes extending in a radial direction, while others have their long axes extending vertically. In tangential view, in which the cross-section of the medullary ray is shown, these differentiated marginal cells are seen to occur as an outside layer of cells, practically investing the more numerous and smaller cells of the medullary ray as a sort of sheath. The rays vary in height from 0.15 to 2.65 mm., and in width from 0.02 to 0.09 mm. at the widest point. The individual cells of the ray, as measured in transverse section, vary in thickness from 7 to 21.3 μ, and from 48 to 105 μ in length, with an average of 65 μ. In height they vary from 35 to 166 μ, with an average of 50 μ.

All the cells of the medullary ray contain numerous simple pits where they join similar parenchymatous cells. Where they join conducting elements such as fibers and tracheids, the pits are half-bordered, being simple on the wall of the ray cell, but bordered on the wall of the conducting elements. The cells of the ray contain numerous large starch grains 8–10 μ in diameter. These have prominent hila. Mucilage is frequently present in the cells of the ray in small irregular masses.

WOOD PARENCHYMA.—In transverse section the wood parenchyma elements appear diffused throughout the growth rings, but are

most numerous around the vessels. In a longitudinal aspect, the wood parenchyma strands appear much like septate wood fibers, differing from these by their thinner walls and numerous, simple pits (fig. 32). The strands usually consist of four cells arranged end to end in vertical rows. The septations are transverse and the individual cells are prismatic, except the end ones, which are pointed. They measure 16–55 μ in width and 40–196 μ in height.

The number of pits in these elements varies. Where they are adjacent to medullary ray cells or vessels, the pits are more numerous than when they lie adjacent to fibers or to other wood parenchyma cells. The wood parenchyma elements are usually in seriation with the vessel segments.

Crystals of the rhombohedral type are common (fig. 29). These occur in chambered parenchyma strands, four to six to the strand. They measure about 20×25 μ. They are not found in material that has been treated with hydrofluoric acid.

FIBER TRACHEIDS.—Fiber tracheids are occasionally found in this wood (fig. 31). When present they usually are found adjoining the vessels. In transverse section they resemble small vessels. They are much thicker and shorter than the wood fibers, measuring about 55×475 μ. The walls are thinner than the wall of either the wood fibers or vessels, and are supplied with prominent bordered pits, not unlike those found in the walls of the vessel. These elements taper to a gently rounded point at the ends.

GROWTH RINGS.—The growth rings are irregular in occurrence, and do not correspond to annual periods of growth. They are in all probability due to a seasonal variation in climate causing a periodic growth in thickness of the stem. The rings exhibit considerable variation in width, and are demarcated by a band consisting of four to six rows of parenchymatous cells. These are continuous through the lignified and unlignified parenchymatous zones, and are to be distinguished from the other parenchymatous elements by a gradual reduction in their radial diameter.

PITH.—The pith is nearly round in transverse section, with a minutely crenulate outline. It averages 7 mm. in diameter, and is made up of isodiametric, polygonal parenchymatous cells whose diameter is about 150 μ. The walls of the cells are thin, from 1.8 to 2 μ in thickness. The pith crown consists of five to eight rows of cells

that are somewhat smaller than the cells toward the center of the pith area. The cell walls have a slight secondary thickening, and are abundantly pitted with simple pits. No lignified cells are present. The pith is distinctly septate when seasoned; the openings between the diaphragms are narrow and extend beyond the inner edge of the pith crown. Mucilage cavities are numerous, often forty or more appearing in one transverse section. Those of the central region are round in transverse section and are arranged in an irregular fashion, while those of the pith crown are elliptical in transverse section and are arranged around the pith in the form of a circle. These cavities measure about 240 μ in diameter, and in most cases are clogged with mucilage. When a portion of the pith was removed and placed in water, strands of mucilage exuded until the water became thick like syrup.

Heliocarpus appendiculatus

Locality and habit of growth

The structure of the wood of this tree is so similar to that of *Heliocarpus popayanensis* that a detailed description is unnecessary, and emphasis will be placed chiefly upon differences. The tree is native to Central America and Mexico. It has been collected in Guatemala by John Donnell Smith, in the State of Tabasco, Mexico, by Linden, and in Talemania, Costa Rica, by Ad. Tonduz. The wood specimen upon which this study is based was collected near San Jose, Costa Rica, in the summer of 1918. The plant differs from *H. popayanensis* in having leaves that are appendaged at the base. The trees have long, straight trunks, 25–30 cm. in diameter. The leaves are not unlike those of basswood in outline. The trees grow gregariously in the low hot lands as well as in the cooler uplands. The tree is called Burio Blanco in Costa Rica, and Macate in Guatemala. The former name means white wood, and the latter refers to the fibrous nature of the bark. These vernacular names are also applied locally to various other plants of the Tiliaceae.

Gross anatomy

The wood of this tree, like *H. popayanensis*, is a light weight, diffuse porous, laminated wood, without pronounced taste or odor. The wood near the periphery weighs 15.8 pounds to the cubic foot,

when oven-dried at a temperature of 100° C. It has a specific gravity of 0.25. The wood near the pith weighs 9.67 pounds per cubic foot, and is much softer and less fibrous in nature than the later formed wood. Ripple marks are readily seen on the surface of the longitudinal cuts, and appear more prominent on the tangential than upon the radial surface. These marks occur on the average of twenty-six to the centimeter. They are caused by the tierlike rankling of the wood parenchyma strands. The wood fibers or cells of the wood parenchyma strands are not in seriation.

Minute anatomy

Vessels.—The microscopic structure of this wood is similar to that of *H. popayanensis*. It has a laminated appearance when viewed in transverse section. It exhibits a similar difference in the type of wood found in the central part of the axis and that toward the periphery. The merging of the medullary rays into the bands of parenchymatous tissue is more noticeable than in *H. popayanensis*. No differences are observed in the size, shape, or markings of the vessels, except that an occasional scalariform perforation is found. In one case a vessel segment was found with such a perforation at one end and a porous perforation at the other (fig. 27). The vessels are fewer than in *H. popayanensis*. The wood fibers differ only in their greater length and slightly greater diameter, as shown by the following average measurements:

	Maximum	Minimum	Average
Length	60 μ	18 μ	37 μ
Width	2.43 mm.	0.756 mm.	1.37 mm.

No important differences are exhibited in the wood parenchyma elements of the two species. The medullary rays fall more clearly into the two types, uniseriate and multiseriate, there being few transitions between the numerous uniseriate and the high multiseriate rays. Mucilage is rarely found, and the presence of crystals has not been observed.

The species exhibits no semblance of annual rings of growth, differing sharply in this respect from *H. popayanensis*. Similar observations have been made in *Ochroma*. The Ecuador species of this genus show pronounced growth rings, while in the Central Ameri-

can species they are lacking. The cause of this phenomenon is probably physiological, and no doubt to be associated closely with the character of the climate of the two regions.

Comparison of outer and inner wood in Heliocarpus

The wood of most trees of temperate regions can be divided into two portions, heart wood and sap wood. It is well known that the former is usually harder, and in general of a darker color than the latter, due to the infiltration of gums, resins, and other substances into the walls of the cells of that region. It is equally well known that sap wood, in such cases, contains the only living elements of the wood, and that, generally speaking, it is less valuable commercially than the heart wood.

The study of two species of *Heliocarpus* has resulted in some divergences from these generalities. Technically, only discolored parts are called heart wood, and in this respect, in the two species of *Heliocarpus* studied, heart wood is lacking. There is, however, considerable difference in the hardness, density, and weight in the wood of the two regions, as is evident from the data given. They show that the wood nearest the periphery is in all cases harder and heavier than that nearer the pith, and that this change is not abrupt. It should be stated, however, that no increase in hardness or weight occurs until the end of the third season's growth.

The problem of accounting for the physiological change undergone, whereby harder, heavier, and denser wood is produced, is difficult of solution without direct experimental evidence. It has recently been pointed out by Kraus and Kraybill (7), however, that a relative decrease of nitrates in proportion to the carbohydrates makes for fruitfulness, fertility, and lessened vegetation. These plants produce flower and fruit about the fourth year, and subsequently their growth is greatly retarded. Histological examinations show that the transition from the softer and lighter to the heavier and harder wood begins to take place about the time the tree flowers and fruits. This suggests that the greater density and degree of lignification of the wood might be due to an upset in the carbohydrate nitrogen relations undergone in the plant at the time of fruiting.

Although finding it difficult to determine any definite proportion

of the lignified and unlignified areas, in general, it can be said that the former become larger and compose more of the bulk of the tissue toward the periphery, while the latter correspondingly increase nearer the pith. These parenchymatous bands are sometimes 2 mm. thick tangentially in this region. They are composed of pithlike, short cylindrical cells, with their long axes extending vertically; while, as has just been shown, the similar areas in the wood near the periphery are composed of unlignified wood fibers. These conditions in the fundamental composition of the two regions account for the more fibrous nature of the wood toward the periphery, and for the absence of "ripple marks" in the wood nearer the pith. Infiltration of gums, mucilage, or other substances into the cell walls has not occurred, but in the region near the pith the lumina of the vessels often contain these substances. True wood parenchyma elements are far more numerous in the region near the periphery, and become fewer and fewer until they disappear entirely near the pith. Numerous measurements were made to ascertain whether the walls of the elements of the two regions showed any differences in thickness, but no noticeable differences were found to occur.

Apeiba aspera

Locality and habit of growth

The wood sample of *Apeiba aspera* was taken by Dr. H. E. Stork in Costa Rica during the summer of 1920. A specimen of the plant bearing fruit and leaves was also taken. The plant belongs to the tribe Apeibeae of the Tiliaceae, and is therefore closely related to *Heliocarpus*. The genus *Apeiba* consists of six arboreous and shrubby species endemic in the hot countries of America. The members of this genus are characterized by the possession of discoid spiny fruit, 6–8 cm. in diameter. The species is widely scattered, having been collected not only in Costa Rica, but upon the Atlantic and Pacific sides of South America as well. It is found more abundantly in Costa Rica in the llanos and hills of the Atlantic side (**9**). It is a large symmetrical tree with a long cylindrical crown, gently rounded at the top. It attains a height of 40 m. and a diameter of 0.7 m. The natives call it Peine de Mico in Costa Rica. It is also known as Burio, but, as has been pointed out, this term is applied indiscriminately to

several species of the Tiliaceae, as well as to some few species belonging to other families.

Gross anatomy

The wood of this tree is a diffuse porous, laminated, light weight wood (fig. 3). It is somewhat finer in texture than the wood of *Heliocarpus*, and similar to the latter in having no sharp line of demarcation between the wood of the central part of the axis and that toward the circumference. The wood near the pith is much softer, lighter, and more extremely laminated than that toward the periphery. There is a greater difference between the two regions than noted in *Heliocarpus*. The wood near the periphery, when oven-dried at a temperature of 100° C., weighs 19.5 pounds to the cubic foot, and has a specific gravity of 0.31; while that near the pith runs as low as 10.13 pounds to the cubic foot and has a specific gravity of 0.16. It is evident from this that the weight of any given block of wood will vary considerably, depending upon the proportion of wood from the two regions which it contains.

The wood is light brown, odorless, and tasteless. "Ripple marks" are numerous on both the radial and tangential surfaces, and can be seen easily with the unaided eye. These ripple marks are more numerous on the tangential than upon the radial surfaces. As noted in *Heliocarpus*, these ripple marks are due to the tierlike ranking of the wood parenchyma strands, and appear to be a constant feature attending all investigated woods of the Tiliaceae. Growth rings are visible to the unaided eye, and are extremely variable in width. The medullary rays do not continue across the parenchymatous zones in the region near the pith, but lose their identity completely as they merge into the parenchymatous tissue of this region. They reappear, however, when the next zone of lignified tissue is reached, and run through this uninterrupted.

Minute anatomy

Vessels.—In a transverse section, as in fig. 3, the vessels are seen to be evenly distributed throughout the wood. These are usually scattered singly, or in radial rows consisting of three or four vessels. The average radial diameter is 250 μ, while the average tangential diameter is 175 μ. The length of vessel segments, as obtained from

measurements of numerous isolated elements, is maximum 0.843 mm., minimum 0.320 mm., with an average of 0.510 mm.

The pores are oval in outline, or in cases where two or more vessels join, the contiguous tangential walls are flattened in a radial direction (fig. 3). The walls of the vessels are 8–12 μ thick, strongly lignified, and are marked by numerous bordered pits (fig. 22). These pits average 32 μ in diameter, and their arrangement and shape are not different from those noted in the vessels of *Heliocarpus*. The cross walls of the vessels are mostly transverse, but an occasional oblique one is found. The terminal apertures are porous and the annular ridges are extremely narrow (fig. 4). Mucilage is present in small globules in some of the vessels.

WOOD FIBERS.—The wood fibers are usually longer than those of *Heliocarpus*. They vary in length from 0.6 to 1.9 mm., and average 1.23 mm. Their average width, at the widest point, is 35 μ. The ends of the fibers are often forked, and sometimes the margin of the fiber toward the end presents a sawlike appearance, due to the pressure exerted by the adjoining medullary ray cells. The longitudinal course of the fibers is greatly distorted, due to the pressure of very "fat" medullary rays (fig. 4). Within the lignified areas the fibers are lignified in various degrees, grading from unlignified fibers to strongly lignified fibers. The thickness of the cell walls of the fibers varies between 5 and 8 μ, the average being about 6.5 μ. Numerous bordered pits are present. These are slitlike and are elongated horizontally (figs. 17, 19). Usually their long axis lies obliquely to that of the fiber. The border is very much reduced and the pits frequently simulate simple pits. A great many septate wood fibers are present. Ordinarily there is not more than one septum to the fiber, but occasionally two or more are present. These measure 2 μ in thickness and stain densely with haematoxylin.

MEDULLARY RAYS.—The enormous medullary rays in this wood are perhaps its most striking character, and are shown to best advantage in a tangential section (fig. 4). In this aspect they are seen to be, in most cases, seven or eight cells wide tangentially. Occasionally a uniseriate ray is found, but these are not numerous. The average height of the rays is 0.83 mm., but rays as high as 2.0 mm. are occasionally found. The cross-section of the ray cells (tangential

section of wood) shows them to be practically alike in size and shape. They are 30 μ in width and are practically circular in outline. The cell walls are about 7 μ thick, and contain numerous circular, simple pits. A radial section of the wood shows to advantage the heterogeneous structure of the ray. The marginal cells have their greatest diameter vertically. In some rays the majority of the cells composing the ray appear to be similar, while in other rays there is a gradual transition between the type of cells of the marginal row, to procumbent cells within the body of the ray. No difference exists as regards the walls, pitting, or contents of the ray cells of the margin and those within the ray, and therefore these cannot be regarded as true marginal ray cells such as are found in *Nyssa*, *Aesculus*, and *Salix*. As ascertained from radial sections, the upright ray cells average 0.066 mm. radially and 0.058 mm. vertically. The procumbent ray cells average 0.087 mm. radially and 0.041 mm. vertically. The ray cells contain numerous hexagonal crystals and tannin granules. The crystals average 24 μ in height and are 17.5 μ wide. The cells contain large nuclei and are rich in other protoplasmic contents.

WOOD PARENCHYMA.—The wood parenchyma is diffuse and is found abundantly throughout the stem. Its strands are 0.3–0.5 mm. in length, and consist of four perpendicular cells, although occasionally a strand consists of six or seven cells (fig. 16). The strands are not in seriation with the vessel segment or wood fibers, neither are the individual cells of adjoining fibers in seriation. The average width of a wood parenchyma cell, tangential diameter, is 22.5 μ. The wall is 3.5 μ thick and abundantly pitted with simple pits. The height of the individual cells of the strand averages 76 μ. The cells retain their protoplasmic contents indefinitely. No crystals are present. The walls of the wood parenchyma cells are sometimes slightly lignified, and are easily confused with the septate wood fibers.

GROWTH RINGS.—The rings of growth in this species are much more distinct than in *Heliocarpus*, but evidently do not represent true annual increments of growth. They are delimited by a single row of wood parenchyma strands, and are very variable in their circumference. They are considerably wider in the central part of the axis, and become gradually narrower toward the periphery.

BARK.—The bark of a small trunk 10 cm. in diameter measures 7 mm. in thickness. It is very tough and fibrous, and is much harder and heavier than the wood. The color is cinnamon brown, and it is covered with small wartlike projections. A transverse section shows it to be filled with large irregular cavities, containing mucilage in profuse quantities. These cavities are sometimes 3 mm. or more in diameter.

Pachira barrigon

LOCALITY AND HABIT OF GROWTH

Pachira (**2**) is a genus of the Bombacaceae, a family noted for the great quantity of light weight wood which it produces. To this family belongs *Ochroma*, which has nine species, all of which produce the light weight wood known commercially as Balsa. *Pachira* includes about forty species, all endemic to tropical America. *P. barrigon* is the largest species of the genus, the trees often reaching a meter or more in diameter. They grow gregariously in Panama and Costa Rica, and are known as the "big bellied" trees, due to the peculiar enlargement of the trunks a few feet above the ground. The plants reproduce freely from cuttings, and are used locally in producing fences and hedges. The wood is used extensively for forms in concrete construction work.

GROSS ANATOMY

This wood is one of the most important of the tropical light weight woods, and resembles "balsa" more closely than the other woods discussed. It is homogeneous, and is composed approximately of 90 per cent parenchyma. It is diffuse porous. The pores are relatively larger and fewer in number than in the other woods studied. The wood is tasteless and odorless, and weighs 10 pounds to the cubic foot when oven-dried. It has a specific gravity of 0.16. "Ripple marks" are visible on the tangential and radial surfaces. These are due to the arrangement of the wood parenchyma strands in radial and tangential seriation, and to a similar arrangement of the cells that compose the strands. These marks are very much smaller and more numerous than those found in the other woods discussed in this paper. There are, on the average, seventy of these ripple marks to the centimeter.

MINUTE ANATOMY

VESSELS.—The vessels as exhibited in a transverse section are oval in outline, with their long axis extending in a radial direction (fig. 5). The average width of the vessels in tangential diameter is 249 μ, and their average width in the radial diameter is 355 μ. The walls vary in thickness in different vessels, but average 7.2 μ. Tyloses and mucilage are lacking in the vessels. The variation exhibited in the length of the vessel segments is maximum 0.498 mm., minimum 0.192 mm., with an average of 0.328 mm.

The pitting in the walls is like that described for *Heliocarpus popayanensis*, except that the closing membrane becomes greatly elongated horizontally, and is very narrow vertically. The pits are so crowded that a hexagonal outline to the border is the rule. The diameters of these pit borders average 10 μ, while the size of the pit mouth is 3.5×6.5 μ. The cross walls of the vessel segments are generally transverse, but occasionally one is found that is slightly oblique. The terminal apertures are always porous, and have pronounced annular ridges.

WOOD FIBERS.—The fibers are arranged irregularly within the parenchymatous tissue (fig. 5). They are variable, often extremely long, ranging in length between 0.664 and 2.59 mm., with an average of 1.23 mm. The average width of the fibers at the point of their greatest diameter is 35 μ, while the thickness of the wall is 7 μ (figs. 24, 25). The pits are reduced bordered pits with elliptical mouths. In some fibers they are greatly reduced in number. The border averages 7 μ in diameter. In macerated material some of the fibers are seen to possess serrated edges and forked ends. The latter are usually needle-like, but are often flattened and bent. The margins of the fibers in many cases are permanently indented, due to the turgidity of the adjoining parenchymatous cells (figs. 24, 25). The indentations are always opposite, because of the seriation, and correspond in their vertical extent to the height of these parenchymatous cells. Septate fibers are frequent in this wood. There is usually but one septum within these fibers, but occasionally there are two or more. These are 1.5 μ in thickness, and have a different chemical nature from the walls of the fiber, as exhibited by their greater affinity for haematoxylin stain.

WOOD PARENCHYMA.—Wood parenchyma makes up the bulk of the wood. As has been pointed out, the wood fibers are irregularly interspersed within this parenchymatous tissue. The wood parenchyma strands are about 0.5 mm. in length, and typically consist of four cells. These cells average 45 μ wide tangentially, and give rise, because of this, to many intercellular spaces. As is typical of wood parenchyma strands, the tip cells taper to a somewhat rounded point, which interlocks between the tip cells of the two adjoining strands. The numerous simple pits are almost circular in outline, and are about 7 μ in diameter. The cell walls are thin, averaging about 3.5 μ. No crystals are present, but many of the cells contain mucilage, tannin granules, and starch grains.

MEDULLARY RAY.—The medullary rays are far less prominent than in *A peiba*. Both uniseriate and multiseriate rays are found; the former are of very limited occurrence, while rays two or three cells wide is the rule (fig. 6). Rays more than four cells wide never occur. The cells of the medullary ray differ somewhat in size and shape, those toward the margin being somewhat shorter radially and wider perpendicularly than those away from the margin. The cell walls are 3.5 μ thick, and contain numerous simple pits 3–7 μ in diameter. In a radial section the cells average 35 μ in height, and vary from 40–125 μ in length. Their third dimension, as ascertained by averaging the measurements obtained from fifty cells in transverse section, is 22 μ. The rays are not in seriation with the wood parenchyma strands, but a considerable number end at the same level, as is shown in tangential section (fig. 6). In this section the height of the individual rays is easily obtained. Some of these attain the height of 1.6 mm., but the average height was 0.830 mm. Large starch grains 8–10 μ in diameter occur profusely in the cells. Cells containing tannin granules are numerous, but crystals are lacking.

GROWTH RINGS.—The growth rings are unusually prominent for a light weight tropical wood, due to the presence of dark brown lines. These appear on the face of the summer wood, and clearly delimit the season's growth. The dark line is due to the greater number of lignified fibers in this region. The growth rings average 1.5 mm. in width near the pith, but toward the periphery they become narrower, and are usually less than 0.5 cm. wide.

Cavanillesia platanifolia

Locality and habit of growth

Cavanillesia is a genus of the Bombacaceae, being closely related to *Pachira* and *Ochroma* (**17**). The genus consists of two species in South America, one in the eastern and one in the western part. *C. platanifolia* extends northward into Panama, eastward into Colombia, and southward into Peru (**10**). The trees have huge, straight trunks, 2–3 m. in diameter, and very small, flat crowns. The few branches are usually given off near the top of the tree. They are called Quipo in Panama, and Maconda in northwestern Colombia. These trees have very light weight wood, which has been proposed for commercial use as a substitute for cork, and as an insulating medium under the name of "quipo" or "cuipo." The bark of the tree appears to be the chief source for mechanical support. This is two or three inches thick and is tough and fibrous. Workmen are very wary about cutting these trees, on account of their immense weight and the difficulty of telling which way they will fall. It is said that if the bark be cut entirely around a tree it will fall of its own weight. There are no data available as to rate of growth, but the histological appearance of the wood indicates that it grows rapidly. The trees are deciduous, the leaves being off in the dry season, December to May. The wood specimen upon which this study is based was collected near Pedro Muguel, Panama, in 1918.

Gross anatomy

The wood of this tree is diffuse porous, soft, light, and weak. It is light yellow, odorless, and tasteless. When green the wood is much heavier than water, but when kiln-dry it is the lightest wood known, with a weight of 6.2 pounds per cubic foot and a specific gravity of 0.10. The wood from the periphery may weigh as much as 11 pounds to the cubic foot. The vessels are relatively large and visible to the naked eye. They are rather regularly distributed, but become more numerous toward the outer limit of the growth rings. The medullary rays are visible without a lens, and appear somewhat lighter in color than the rest of the wood. They are very large and prominent on the radial surface. Growth rings are prominent and are limited by a

light colored line. Very fine indistinct "ripple marks" are present on the longitudinal surfaces. These are caused by the horizontal seriation of the parenchyma cells. The medullary rays are not storied, but there is a tendency for the vessel segments to appear in seriation.

Minute anatomy

Vessels.—In transverse section the vessels occur singly or grouped into clusters containing two to seven vessels. When single the vessel is oval in outline, averaging 200 μ in diameter tangentially, and 350 μ in diameter radially (fig. 11). The average thickness of the wall is 6 μ. The wall is heavily lignified and richly supplied with bordered pits measuring 11 μ in diameter. The pit mouth measures 3×10 μ, and has its long axis horizontally. These pits are arranged alternately, and usually have a circular border, which, however, often becomes hexagonal by mutual compression. Where the vessel adjoins the cells of the medullary ray the pits are half-bordered. The vessel segments are simple porous with nearly transverse cross walls. The annular ridge is not prominent, usually being less than 6 μ wide. Measurements taken of the length of fifty vessel segments gave a maximum of 0.750 mm., a minimum of 0.640 mm., with an average of 0.675 mm.

Wood fibers.—The wood fibers are very few, and are generally isolated in and completely surrounded by parenchymatous tissue (fig. 11). They are more lignified, much smaller in diameter, and have much thicker walls than the parenchyma cells by which they are surrounded. In a transverse section it is very unusual to find two or more of these fibers with contiguous walls. The condition of the fibers in their relation to the surrounding parenchyma is similar to that noted in the wood of *Pachira*, except that the fibers are many times more numerous in the latter wood, as is evident by a comparison of figs. 5 and 11. The outline of the fibers in transverse section is hexagonal. Their average diameter is 25 μ, and their average length is 0.85 mm.

The distribution and relative bulk of the fibers in relation to the parenchyma tissue are interesting, and in this regard numerous counts were made. A transverse section was divided into areas 10 mm. square, and counts of the fibers appearing in each square gave

minimum 0, maximum 28, and average 16. From these data it is apparent that the number of fibers is extremely variable in different areas of the stem, and that they never make up more than a very small proportion of the bulk of the wood.

Reduced bordered pits are present in the walls of the fibers. These are slitlike, 1.5×6 μ in diameter, and their long axis is oblique to that of the fiber (fig. 21). Upon the inner walls of the fibers definite spiral or scalariform thickenings are present. These do not extend the length of the fibers, but occur here and there along its axis. The fibers are usually septate, and never appear in the parenchymatous sheath immediately surrounding the medullary rays.

WOOD PARENCHYMA.—The wood parenchyma is paratracheal, and is composed typically of four cells arranged perpendicularly into a strand. These cells are usually flattened radially. Their average is 15×45 μ, and their height 60 μ. The cell wall measures 2 μ, and the numerous simple pits marking the wall average 3.5 μ in diameter. Plain parenchyma, similar to the wood parenchyma, but with much larger cells, makes up the bulk of the wood. In a transverse section these cells are usually hexagonal and have a greater radial than tangential diameter, measuring 63×105 μ respectively. The walls measure 1.5 μ in thickness, and contain an occasional small simple pit 3.5 μ in diameter. In a longitudinal section these cells resemble pith cells. They average 128 μ in height (fig. 12). Microchemical tests for lignin showed, contrary to expectation, that the walls of these cells are slightly lignified throughout. The light weight of the wood is to be correlated with the large lumina of these parenchyma cells, their thin walls, and their relatively great numbers, together with the relatively small number of wood fibers.

MEDULLARY RAY.—The medullary rays are usually multiseriate, but an occasional uniseriate ray occurs. In transverse section they are rather regularly distributed, but are variable in width throughout their course, appearing multiseriate, then uniseriate, or perhaps disappearing entirely, only to reappear some distance beyond. On either side of the medullary ray is a parenchymatous zone two or more cells wide, in which no wood fibers are to be found. In tangential section the estate of these cells is more easily seen. They surround the smaller cells of the ray as a sheath, the outside margin of

which is distinctly spindle-shaped, and which is usually bounded by the slender wood fibers which weave between the spindle-shaped masses (fig. 12). The parenchyma cells of the sheath often pass into the ray, making its margin very irregular and often dividing it into two or more areas. In a radial view the ray is seen to be heterogeneous, and appears very extensive, making up approximately 50 per cent of any given area.

The ray cells are much smaller than the parenchyma cells of the sheath, and have somewhat thicker walls. As ascertained from a tangential section, the cells average 35 μ in vertical diameter and 22 μ in tangential diameter. The simple pits in the walls of the medullary ray cells average 5 μ in diameter. The ray cells contain starch, while the cells of the sheath often contain mucilage in profuse quantities.

GROWTH RINGS.—The growth rings are prominent, and are limited by several rows of parenchymatous cells that are narrower radially and generally smaller than the cells of the other portions of the ring (fig. 11).

Wercklea insignis

LOCALITY AND HABIT OF GROWTH

Wercklea is a genus of the Malvaceae, recently established by PITTIER and STANDLEY (**19**). It is a member of the subfamily Hibiscaceae, and is most closely related to *Hibiscus*. *W. insignis* is the only species of the genus, and is confined to the northwest slope of the Central Cordilleras in Costa Rica, the type locality being the pass known as La Palma, between the volcanoes of Irazu and Barba. The largest specimens are said to grow on the lower grounds north of and below the pass. It has large showy flowers resembling those of the hollyhocks. A few specimens have been planted as ornaments on the grounds of Dr. JIMENEZ in Santa Jose, Costa Rica. The flowers are ephemeral, usually fading the day they blossom. For this reason the natives call the tree "Flor de dia." The tree reaches a height of 10 m., and has a trunk 30–40 cm. in diameter. Our specimen was collected in the type locality in 1918, and according to the collector the tree appears to be a rapid grower, but is not abundant. The collector tried repeatedly to germinate the seeds of this plant but was unsuccessful.

Gross anatomy

The wood of this tree is a homogeneous light weight wood with unusually fine texture. It is ivory colored, odorless, and tasteless. When oven-dry it weighs 15 pounds to the cubic foot and has a specific gravity of 0.24. The pores are irregularly distributed throughout, but appear to be more numerous in some sections of the stem than in others. No growth rings are present, and there is no differentiation in color between the heart wood and sap wood. The latter, however, is somewhat lighter than the former. The medullary rays are visible to the unaided eye, are rather fine, and somewhat lighter in color than the rest of the wood. "Ripple marks" are visible on the radial surface. There are approximately fourteen of these to the centimeter, and the distance between the marks is fairly constant. The bark is thick, tough, and fibrous.

Minute anatomy

Vessels.—The vessels are few in number and are distributed irregularly throughout the wood. In a transverse section of the wood the pores appear oval in outline, with their greatest diameter in a radial direction. Often four or five of these pores appear aggregated into a cluster with no regular arrangement (fig. 7). The walls average 4 μ in thickness, and are more strongly lignified than the walls of the wood fibers. The numerous bordered pits on the vessels are arranged alternately. The borders are generally circular, but often become hexagonal by mutual contact. The diameter of the pit border is 10–12 μ, while the mouth of the pit, which is elliptical, measures 7×3.5 μ. The fusion of these pits in some vessels gives rise to a scalariform appearance, and in other vessels the borders disappear and give rise to simple pits, extremely variable in size. The vessel segments are unusually short, varying in length between 0.3 mm. and 0.6 mm. in radial diameter. They average 0.24 mm., and in tangential diameter 0.144 mm.

Wood fibers.—The fibers in this wood are its most striking character (figs. 20, 33). They differ in many respects from the fibers of the other woods investigated. The walls are very thin, averaging 3.5 μ, weakly lignified, and contain numerous reduced bordered pits,

which are elliptical and about 4 μ in diameter. The fibers are extremely short and stubby. Their average width is 60 μ and their average length is 0.8 mm. It is not unusual to find fibers that are 0.1 mm. or more in width. They are more or less uniform in width throughout their greater length. In most cases they do not have long tapering ends, but come more sharply to a rounded point. The fibers are not definitely storied, but a tendency toward such a condition is evidenced in radial view. In transverse section the lumina are very irregular in size and shape. The outline of the fiber is usually hexagonal (fig. 7). Macerated material often shows fibers with serrate margins and forked ends.

MEDULLARY RAY.—The medullary rays are very similar to those described for *Heliocarpus*. Both uniseriate and multiseriate rays are present, the former occurring more frequently than the latter. The multiseriate rays are never more than 6-seriate. In a tangential section (fig. 8) they appear to be composed of cells of about the same diameter tangentially and vertically. These are circular in outline, and when the face wall appears in the section they seem to be abundantly supplied with simple pits 1.9 μ in diameter. The broad rays may be 2 mm. in height. They are not usually multiseriate throughout their entire height, however, but trail off into long uniseriate ends. Not infrequently these uniseriate portions connect the ray with similar multiseriate rays above or below. By a study of the radial section the ray is seen to be heterogeneous. The upright cells average 52×166 μ, while the average of the procumbent cells is 60×112 μ. The average thickness of the cell walls is 4 μ. The cells do not contain starch, mucilage, or other contents.

WOOD PARENCHYMA.—The parenchyma is chiefly of the paratracheal type, but is also quite abundantly diffused throughout the wood. Each strand is composed typically of two perpendicular cells, which measure approximately 60 μ in diameter and 256 μ in height (fig. 30). The wood parenchyma strands are in seriation; this is especially noticeable in radial section, where the septations of the strands form a broken line across the section in a radial direction. This line serves to make the "ripple marks" stand out very prominently on the radial surface. The cell wall averages 4 μ in thickness,

and is richly supplied with simple pits which are approximately 2 μ in diameter. No crystals or other contents are found in the wood parenchyma cells.

Cordia heterophylla

Locality and habit of growth

Cordia is a genus of the Boraginaceae, subfamily Cordioideae. Its species are common and widely distributed in the tropical regions of both the eastern and western hemispheres, more than two hundred species having been described for the genus. *C. heterophylla* was described by Römer and Schultz from material collected in French Guiana. The tree from which the wood specimen for this study was taken was found in the La Palma Pass, Costa Rica, between the volcanoes Barba and Irazu. It was a tree 30 cm. in diameter. It is not abundant and probably is not of great economic importance. The vernacular name applied to the plant by the natives is Zopilote.

Gross anatomy

This is a light weight, laminated, straight grained, diffuse porous wood. The wood is odorless, tasteless, and light brown. Growth rings are visible, but they are very irregular in width. The medullary rays are fine and rather uniform in size and distribution. The pores often appear in prominent tangential rows. These are connected by tangential bands of wood parenchyma that are clearly visible to the unaided eye. There is no apparent differentiation between the wood of the central portion of the stem and that toward the periphery in color, weight, or density. The wood when oven-dry weighs 17 pounds to the cubic foot, and has a specific gravity of 0.27. No "ripple marks" are present on either the tangential or radial surfaces. The bark is light brown, and is extremely tough and fibrous. It is noticeably much heavier and harder than the wood.

Minute anatomy

Vessels.—By a study of a transverse section, such as is shown in fig. 9, the pores of the vessels are seen to be irregularly distributed, but appear to be more numerous within the zone of the last formed wood of each growth ring. The vessels appear singly, or more commonly aggregated into groups consisting of 2–8 vessels that are vari-

able as to size and shape. In case the vessel appears singly, the transverse outline is nearly circular. The walls of the vessels average 5 μ in thickness, and are abundantly supplied with bordered pits. These are arranged alternately, and in most cases have elliptical borders. The borders average 4.5×8 μ in diameter, and surround similar elliptical pits 2×6.7 μ in diameter. The pits have their greatest diameter in a horizontal direction. The tendency of the bordered pits of the vessels to lose their borders, thereby giving rise to simple pits, has not been observed in this wood. The vessel segments are always simple porous, and are extremely short in proportion to their width. The nearly transverse cross walls have almost disappeared, leaving only extremely narrow annular ridges, often less than 7 μ wide. The results obtained from the measurements of fifty vessel segments were as follows:

	Maximum	Minimum	Average
Length	365 μ	100 μ	276 μ
Width	330 μ	100 μ	224 μ

WOOD FIBERS.—A transverse section shows the lignified wood fibers in isolated groups usually surrounded by parenchymatous tissue (fig. 9). These groups are more or less rectangular in outline, and have a fairly definite arrangement in bands, both in a tangential and a radial direction. This is due to the blocking out of these groups by the crossing, at right angles, of the broad medullary rays with the prominent tangential bands of wood parenchyma. A detailed examination of any one group of wood fibers, in transverse section, shows that the lumina of the fibers vary from extremely large to very minute ones. This is due to the fact that the fibers are not storied, so that a transverse section not only cuts the enlarged portion of some fibers, but the tapering smaller ends of many fibers also. These small openings shown among the lumina simulate intercellular spaces, but are readily distinguished from the latter by the fact that their walls are secondarily thickened. The fibers vary considerably in the degree of lignification of their walls, some appearing extremely lignified and others less so. The fibers vary from circular to hexagonal in outline. In general they are circular near the enlarged middle portion, but become hexagonal along the tapering ends (figs. 23, 24). The bordered pits are not numerous. They often appear in isolated

groups, usually near the widest part of the fiber. They measure 2.4 μ in diameter, their long axes being slightly oblique to the long axis of the fiber. The average results obtained from the measurements of fifty wood fibers were as follows: width of fiber at point of greatest diameter 43.3 μ; length of fiber 913 μ; thickness of cell wall 5 μ.

MEDULLARY RAY.—The medullary rays are in most cases 3, 4, or 5-seriate. Uniseriate rays are occasionally found. In tangential section the rays are distinctly spindle-shaped, with bluntly tapering ends. The individual cells of the ray in this section are hexagonal in outline (fig. 10). The cell walls average 5 μ in thickness, and contain numerous simple pits 1.5 μ in diameter. The rays are often 2 mm. or more in height, but average 1.12 mm. The average diameter of the cells of the medullary ray in tangential section is 25 μ. In transverse section the rays appear to be similar to those observed in the other light weight woods, and are heterogeneous in nature. The vertically elongated cells average 55×58 μ and the procumbent cells average 34×115 μ. In transverse section the cells of the medullary ray are often wider tangentially than radially. This unusual condition has been reported previously by SOLEREDER (**18**) in the woods of *Durio*, *Boschira*, and *Neesia*. Many small globules of mucilage occur in the cells of the medullary rays.

WOOD PARENCHYMA.—Wood parenchyma occurs abundantly, forming broad zones around, and tangential bands between, the vessels. These bands, composed largely of wood parenchyma alternating with the zones of lignified wood fibers, give the wood its characteristic laminated appearance. The wood parenchyma strands are usually hexagonal in transverse section, but are often flattened in a radial direction. They average 25 μ in diameter. In longitudinal section the wood parenchyma strands appear to be made up of two perpendicularly placed cells with gently tapering ends (fig. 28). These cells average 0.2 mm. in length. Their walls are 3.5 μ thick and contain numerous circular simple pits 2 μ in diameter. The cells contain many small globules of mucilage. The strands of individual cells of the strands are never in seriation.

General observations

Of the woods studied, *Pachira barrigon* more closely resembles balsa. Its fibers are lignified to a greater degree than *Heliocarpus*,

Apeiba, *Ochroma*, or any of the other tropical light weight woods which the writer has examined. This, together with the fact that the fibers are extremely long, and are often interlocked, would account for the seemingly greater strength and rigidity of form than exhibited by many of these other woods. This would tend to make it a more desirable wood than *Ochroma* for commercial purposes, where strength plus lightness is desired. It consists of approximately 90 per cent parenchymatous tissue, with extremely thin walls and large lumina, factors that have made balsa famous for its light weight, and at the same time have made it excellent material for insulating and buoyant products.

The anatomical composition of this wood indicates that it is equal to balsa in these respects, and, in addition, that weight for weight it is a much stronger wood. The importance of this can be realized better when it is known that for many purposes it is necessary to veneer balsa with a heavier wood to increase its strength, and to treat it with various chemicals to ward off attacks of microorganisms. The writer believes that in the wood of *Pachira* the desired weight can be obtained, together with a stronger and more enduring wood. These factors, when considered with the facts that this wood is available in large quantities, that the tree is a rapid grower, and that the species reproduces freely from cuttings, indicate that this tree should become of great commercial importance as the demand for light weight timber increases.

Summary and conclusions

1. The commercial importance of light weight woods was demonstrated during the recent war. The economic use of these woods is increasing, as their value for insulating refrigerator cars, cold storage plants, and iceboxes becomes recognized.

2. Trees producing light weight wood appear to be confined to certain geographical areas. These areas in general are the continuously hot and humid regions of Central and South America. The trees appear in greater numbers and obtain their best growth in Costa Rica and Panama.

3. Taxonomically the majority of trees producing light weight wood are closely related, in nearly all cases belonging to the Tiliaceae, Malvaceae, and Bombacaceae of the order Malvales.

4. The woods of seven species of light weight timber producing trees are treated histologically. These species are *Heliocarpus popayanensis*, *Heliocarpus appendiculatus*, and *Apeiba aspera* of the Tiliaceae; *Wercklea insignis* of the Malvaceae; *Pachira barrigon* and *Cavanillesia platanifolia* of the Bombacaceae; and *Cordia heterophylla* of the Boraginaceae.

5. Two distinct types of wood are found among the tropical light weight woods, namely, laminated and homogeneous. Of the woods discussed, the former type is represented by *H. popayanensis*, *H. appendiculatus*, *A. aspera*, and *C. heterophylla;* while the latter type is represented by *P. barrigon*, *W. insignis*, and *C. platanifolia*.

6. Histological evidence indicates that the homogeneous type of wood is better adapted for insulation products than the laminated type.

7. The wood of *Ochroma* has been the principal wood used for these products during the past few years. The histological examination of the wood of *Pachira barrigon* supports the conclusion that this would make an excellent substitute. The tree grows rapidly, attains an immense size, and reproduces freely from cuttings.

8. The woods of *Heliocarpus popayanensis* and *Apeiba aspera*, because of their greater specific gravity, fibrous nature, and laminated structure, are less valuable for buoyant materials than *Ochroma* or *Pachira*, and could not be obtained in such quantities.

9. *Cavanillesia platanifolia* produces wood known as "Quipo." This is the lightest wood known and will no doubt become of great economic importance as the demand for light weight wood increases. Trees 2 m. or more in diameter are available in almost unlimited quantities.

10. In the laminated light weight woods investigated, there is a decided tendency for the medullary rays to merge imperceptibly into the areas of parenchymatous tissue, but they regain their identity when the next lignified zone is reached and pass through this uninterrupted.

11. *Wercklea insignis* and *Cordia heterophylla* produce light weight wood of excellent quality, that have a specific gravity low enough to make them suitable for buoyant products. The trees, although not abundant, grow rapidly, and could be produced in a comparatively short time.

12. A distinct difference between the wood produced near the pith and that nearer the periphery is noted in all species investigated: the latter being harder, heavier, and generally more fibrous than the former.

13. The walls of the individual elements do not vary in thickness in different regions of the stem; but the thicker walled lignified wood fibers become relatively more abundant toward the circumference, while the number of thinner walled parenchymatous elements correspondingly increases toward the pith.

14. A correlation between the upset in the carbohydrate-nitrogen relation at the time of fruiting and the differences in density and weight of the wood produced in different areas of the stem is suggested.

15. Light weight woods are fundamentally very similar to heavier dicotyledonous woods. They differ from the latter principally in being composed of thinner walled elements with proportionally larger lumina, a characteristic which accounts for their light weight and low specific gravity.

16. The following characteristics appear to be common to all species producing light weight wood: (1) they are plants with very large leaves that are soft in texture; (2) the bark is thick, strong, and fibrous; (3) the wood is white or very light in color.

Grateful acknowledgment for assistance and suggestions is made to Professor W. W. ROWLEE, under whose immediate direction the work was performed, and to Professors D. REDDICK, H. D. REED, and S. N. SPRING, for help and criticism in the preparation of the manuscript.

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EXPLANATION OF PLATES XXVI–XXVIII

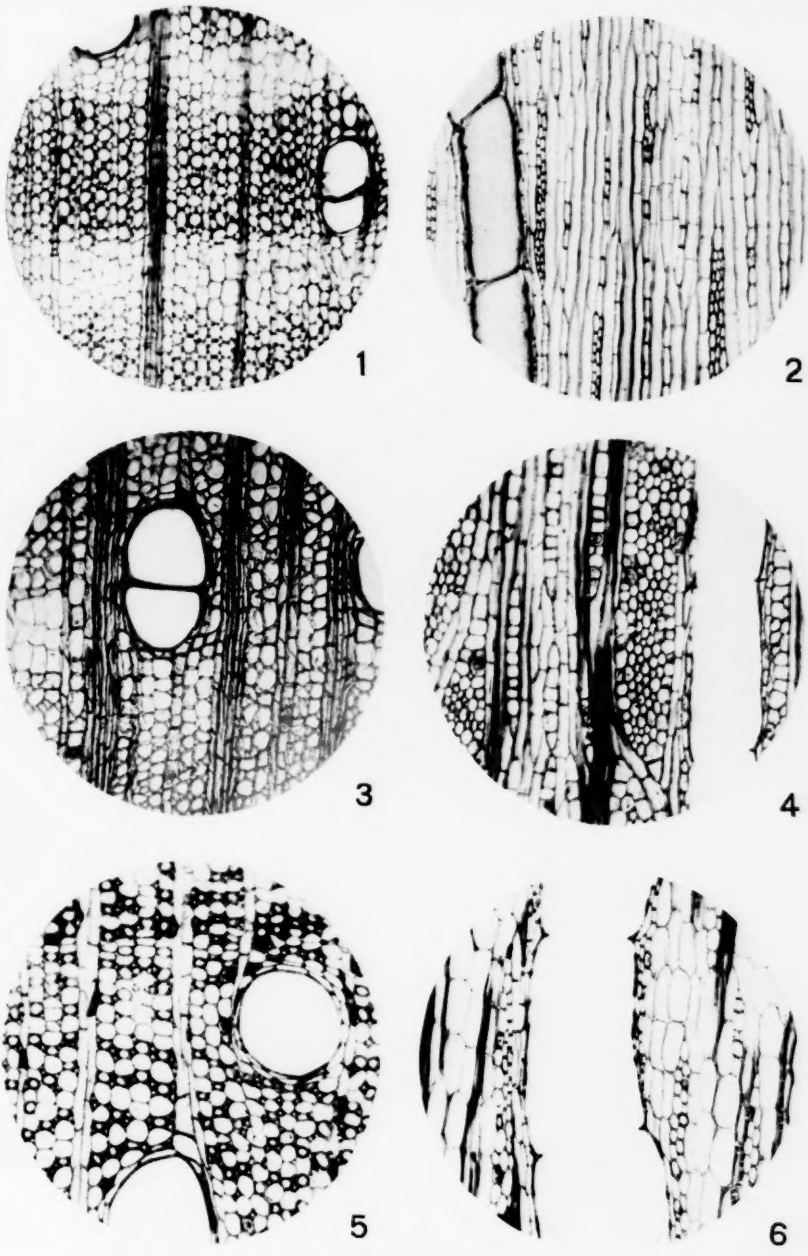
All photomicrographs show magnification of 50 diameters.

PLATE XXVI

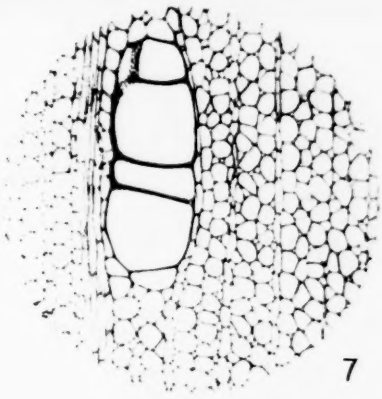
FIG. 1.—Transverse section of wood of *Heliocarpus popayanensis*.
FIG. 2.—Tangential section of same.
FIG. 3.—Transverse section of wood of *Apeiba aspera*.
FIG. 4.—Tangential section of same.
FIG. 5.—Transverse section of wood of *Pachira barrigon*.
FIG. 6.—Tangential section of same.

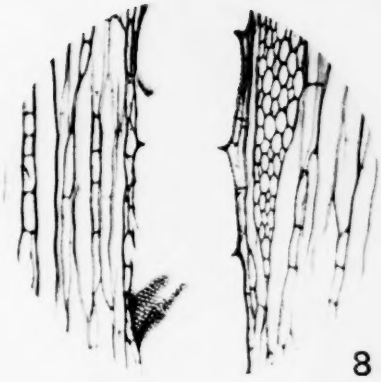
PLATE XXVII

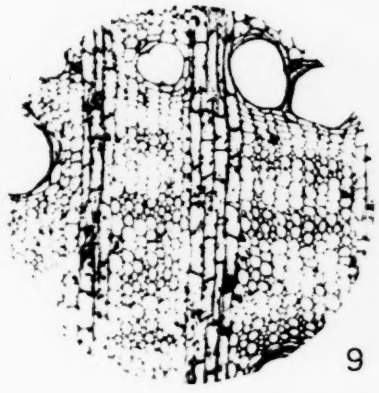
FIG. 7.—Transverse section of wood of *Wercklea insignis*.
FIG. 8.—Tangential section of same.
FIG. 9.—Transverse section of wood of *Cordia heterophylla*.
FIG. 10.—Tangential section of same.
FIG. 11.—Transverse section of wood of *Cavanillesia platanifolia*.
FIG. 12.—Tangential section of same.

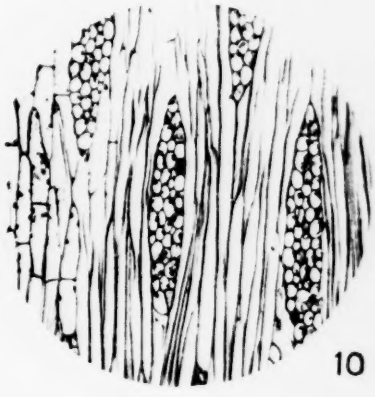


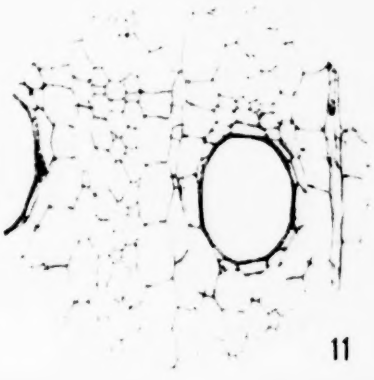
HYDE on LIGHT WEIGHT WOODS

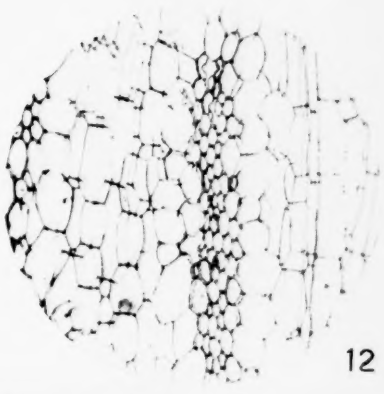




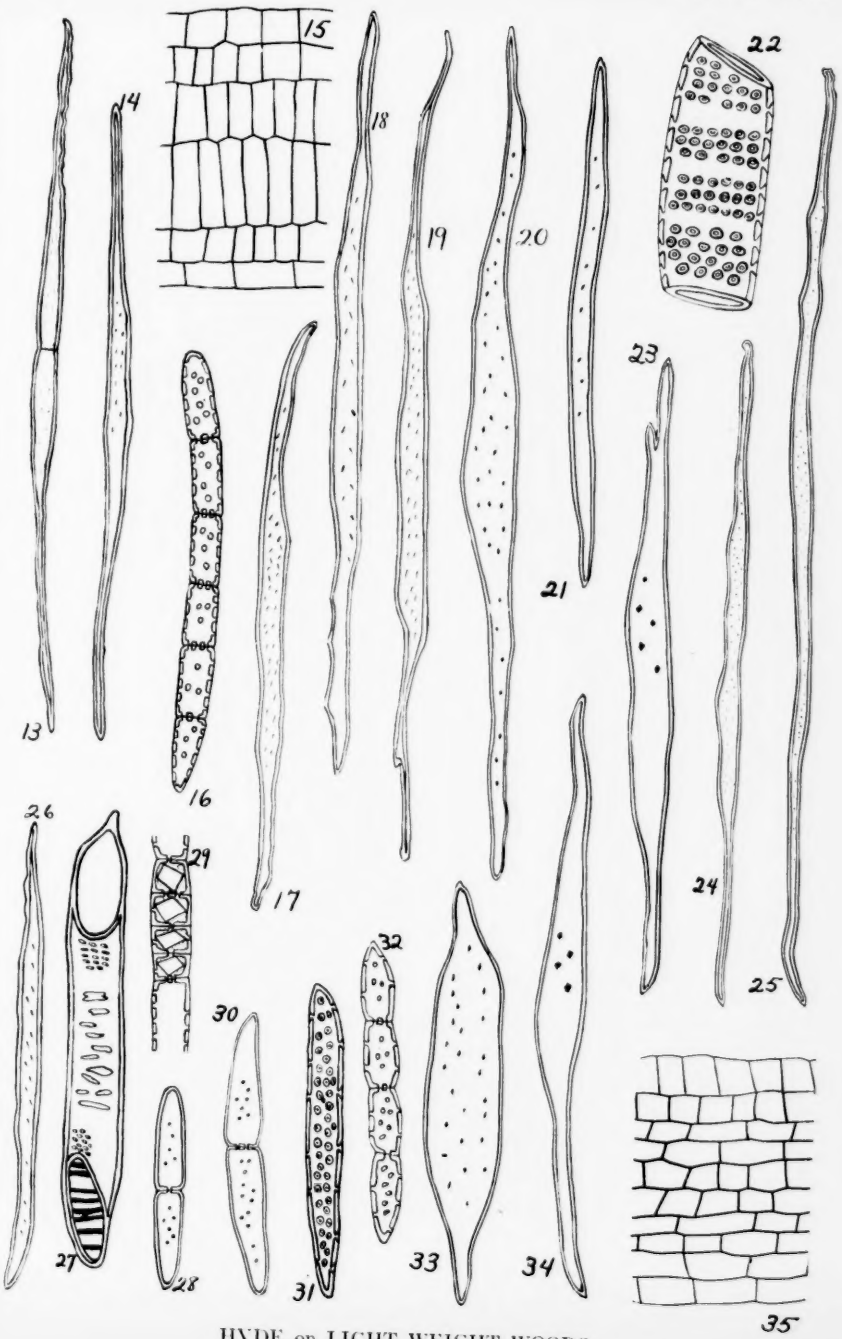








HYDE on LIGHT WEIGHT WOODS



HYDE on LIGHT WEIGHT WOODS

PLATE XXVIII

All outline drawings represent a magnification of 100 diameters.

FIGS. 13, 14.—Isolated wood fibers from wood of *Heliocarpus appendiculatus*.

FIG. 15.—Radial section of medullary ray of *H. appendiculatus*.

FIGS. 17, 19.—Isolated wood fibers from wood of *Apeiba aspera*.

FIGS. 18, 26.—Isolated wood fibers from wood of *H. popayanensis*.

FIG. 27.—Isolated vessel segment from wood of *H. appendiculatus*, showing both scalariform and porous perforations.

FIG. 29.—Portion of wood parenchyma strand, from wood of *H. popayanensis*, showing crystals.

FIG. 31.—Isolated fiber tracheid from wood of *H. popayanensis*.

FIG. 32.—Isolated wood parenchyma strand from wood of *H. popayanensis*.

FIGS. 20, 33.—Isolated wood fibers from wood of *Wercklea insignis*.

FIG. 30.—Isolated wood parenchyma strand from wood of *W. insignis*.

FIGS. 24, 25.—Isolated wood fibers from wood of *Pachira barrigon*.

FIG. 35.—Radial section of medullary ray of *P. barrigon*.

FIGS. 23, 34.—Isolated wood fibers from wood of *Cordia heterophylla*.

FIG. 28.—Isolated wood parenchyma strand from wood of *C. heterophylla*.

FIG. 21.—Isolated wood fibers from wood of *Cavanillesia platanifolia*.

FIG. 22.—Isolated vessel segment from wood of *Apeiba aspera*.

FIG. 16.—Isolated wood parenchyma strand from wood of *A. aspera*.

VARIATION IN SIZE AND FORM OF PYRUS SEROTINA

A. KIKUCHI

(WITH ONE FIGURE)

In connection with fertilizer experiments, the writer studied the effect of applications of different combinations of elements upon variations in the form of the fruits of the Chojuro variety of Japanese pear, *Pyrus serotina* Rehder. The effect of the growing season upon the size and form of the pears was also studied.

The fertilizer experiments under the plans shown in table I were started in 1909, at the Kanagawa Agricultural Experiment Station

TABLE I

Name given to plot	Element	Number of trees
NPK	Nitrogen, phosphorus, and potash	6
NP	Nitrogen and phosphorus	6
NK	Nitrogen and potash	6
PK	Phosphorus and potash	6
N	Nitrogen alone	6
P	Phosphorus alone	6
K	Potash alone	6
nf	No fertilizer (check)	3

near Yokohama. The writer took charge of the work in 1916. For three years previous to 1917 only the annual yield of three trees in each plot was recorded.

The experimental plots contained eight rows and forty-five trees. The trees in each plot and the rows of trees of adjacent plots were twelve feet apart. For three years previous to 1913, farm yard manure, soya bean cakes, wood ashes, sulphate of ammonia, superphosphate of lime, and sulphate of potash were applied to help the normal growth of the trees in all plots except the check. The quantities of fertilizer elements per acre given to the trees during these years are shown in table II.

Since 1913, commercial fertilizers alone were applied to the plots, and the trees in each plot were given either a single element or a combination, corresponding to the experimental plan shown in table I. Nitrogen in the form of sulphate of ammonia, phosphoric acid as phosphate of lime, and potash as sulphate of potash were applied. The annual rates of application per acre during the years 1913 to 1920 inclusive are given in table III.

The figures in tables II and III are computed in English measure, and therefore for the most part are fractional. The reason for this is that the Japanese unit was used for weight and area of land, and was

TABLE II

QUANTITY (IN POUNDS) OF FERTILIZERS RECEIVED BY PLOTS PREVIOUS TO 1913

Plot	1910			1911			1912		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
NPK	67	67	67	83	83	83	90	90	90
NP	67	67	17	83	83	17	90	90	17
NK	67	17	67	83	17	83	90	17	90
PK	17	67	67	17	83	83	17	90	90
N	67	17	17	83	17	17	90	17	17
P	17	67	17	17	83	17	17	90	17
K	17	17	67	17	17	83	17	17	90
nf									

then recalculated to the English unit. Applications of fertilizers were made annually in the early spring, and were well spaded into the ground. No cover crops were grown on any of the plots. The trees were annually cultivated twice in the dormant period, and three or four times in the active period.

The trees were trained on a horizontal trellis 5 feet 5 inches high, the so-called Japanese "tana," which is the most widely used system of training in Japanese pear culture (fig. 1). The trees were planted so closely that the roots of one tree often penetrated the soil of adjacent plots. Previous to 1916 this factor was not considered, but in the fall of 1916 the boundaries of each plot except the check were fixed by thick planks set into the ground to a depth of 2 feet 4 inches. Root pruning was given all trees at the same time. The boundaries of the check plot were boarded after the leaf-fall of 1917. At the same time each plot was trenched along the boundaries, and

roots which had grown through the cracks in the planks were cut. Subsequently the boundaries have been strictly determined every year.

Effect of fertilizers upon form of fruits

SHAW (**2, 3, 4**) reported his study on variation in the form of fruits as follows:

> Some trees showed slight individuality in the amount of variability, and this may be correlated with size, the larger the apples the more variable. This is not true as between the different parts of the tree. As with size, some trees showed quite constant individuality in form of fruit, while others were variable. There seems to be no strong evidence that individuality in size and form is to be found in the tree. The apples from the upper south parts of the tree, which were largest, were also constantly the most flattened.

SHAW also observed a relation between variation in the form of fruits and the climatic condition. His statement is as follows:

> There is a pretty constant relationship between the form of the apple and the temperature for a period following bloom; the cooler this period the more elongated the apple. An effort to delimit this period from the sixth to the sixteenth day following full bloom fits the observed fluctuation in form more closely than any other.

YOUNG (**7**) makes the following statement:

> Some varieties remain quite constant in shape under different conditions, while others are much more plastic in this respect. The most frequent and conspicuous modification of form consists of the elongation of the axis of the fruit relative to the horizontal diameter. This is due to a diminished circulation of the sap caused by change in temperature, giving rise to an insufficient supply to provide for simultaneous development of the fleshy portion and elongation of the axis. Two or three weeks after blooming the form of the fruits becomes fixed and is not noticeably influenced by the moisture supply thereafter.

Variation in the form of the Ben Davis apple in Japan was studied in 1914. On a single tree the form was distinctly elongated in fruit produced on the terminal bud of the long, one-year old shoots, as compared with that on the spurs. The long shoots were divided into two groups in such a way that one group included those more than one foot long, and the other those less than one foot in length. The fruit from the former was always more elongated than that from the latter. The writer also made observations on the relation of the variation in form to the climatic condition in different localities. The

TABLE III

QUANTITY PER ACRE OF FERTILIZERS (IN POUNDS) RECEIVED BY PLOTS SUBSEQUENT TO 1913

Plot element	1913			1914			1915			1919 (16, 17, 18, and 19)			1920		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
1. NPK	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200
2. NP	100	100	.....	133	133	.....	167	167	.....	183	183	.....	200	200	.....
3. NK	100	.....	100	133	.....	133	167	.....	167	183	.....	183	200	.....	200
4. PK	.....	100	100	.....	133	133	.....	167	167	.....	183	183	.....	200	200
5. N	100	.....	.....	133	.....	.....	167	.....	.....	183	.....	.....	200	.....	.....
6. P	.....	100	.....	.....	133	.....	.....	167	.....	.....	183	.....	.....	200	.....
7. K	.....	.....	100	.....	.....	133	.....	.....	167	.....	.....	183	.....	.....	200
8. Check	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE IV

ORDER IN RANK OF ANNUAL AVERAGE VALUE OF FORM INDEX FOR EACH PLOT

Year	Order in rank							
	1	2	3	4	5	6	7	8
1918	nf (122.01)	K (121.71)	P (121.47)	NPK (121.17)	PK (121.10)	NK (120.77)	N (120.60)	NP (120.54)
1919	K (119.87)	PK (119.59)	NK (119.11)	nf (119.00)	P (118.36)	N (118.22)	NP (118.07)	NPK (118.06)
1920	K (121.01)	PK (120.76)	NK (120.60)	P (120.19)	nf (119.86)	NPK (119.59)	N (119.39)	NP (118.90)

summer temperature for three months showed a marked influence on the form of the fruit; that is, the higher the temperature the more flattened the apple.

The Chojuro pear was studied to determine whether there were more or less definite variations in form due to the application of different combinations of fertilizer elements. As stated, the trees were

FIG. 1.—Method of training pear trees on horizontal overhead trellis, the so-called Japanese "tana."

trained on horizontal trellises (fig. 1), and nearly all the fruit buds were produced on spurs. It therefore appears that there should be little or no variation in the form of fruits caused by their being on different parts of the tree, or by length of the bearing shoots. The Chojuro fruit is apple-shaped, rather round and regular, with a shallow cavity and basin.

The method employed to determine the form of the fruit was to record the largest transverse and longitudinal diameters of each individual fruit. These measurements were made with vernier cali-

pers reading to a tenth of a millimeter. The "form index" for the shape of each pear was obtained by dividing its transverse by its longitudinal diameter and multiplying the quotient by 100. The larger the form index the more flattened were the pears, and vice versa. The class value of the form index was made in ranges of 3, and the series of the class and the frequency of distribution were arranged as follows: class $\underset{2}{101}$ (99.5–102.4), $\underset{5}{104}$ (102.5–105.4), $\underset{13}{107}$ (105.5–108.4) (frequency of distribution is shown under each class value). The average value of the form index was calculated by the same method as used in determining the average weight of the fruits. The average values for the form index as obtained in 1918, 1919, and 1920, and the order in rank of the plots are given in table IV.

The range of variation in the form of the fruit caused by different combinations of fertilizer elements is not so wide as the range of variation in size. The extreme difference in the average index between the plots amounts to a trifle less than two in any one season, while between two individual trees in the same plot the difference was sometimes above two, although not often.

There appeared to be some relationship between the size of the fruit and its form. To obtain data the fruits from each of three trees of the Chojuro variety growing in different parts of the same orchard, and receiving fertilizers, were studied in 1919. The coefficient of correlation was calculated to determine the relationship between size and form for each of the three trees. The results were as follows:

Tree no.	Number of fruits observed	Coefficient of correlation
1	244	$+0.009 \pm 0.068$
2	152	$+0.033 \pm 0.081$
3	237	$+0.007 \pm 0.065$

The coefficient of correlation is positive for each, but so small as not to surpass its standard error in any of the three trees. The results show that there is no noticeable correlation between the form and the size of fruits produced on an individual tree. By comparing data pertaining to the effect of fertilizers upon size of fruits with table IV, it was determined that there is no definite relationship between the form of the fruit and its absolute size, even when fluctuation in size is caused by different combinations of fertilizer elements.

Table IV shows that the annual variation in the average index for each plot is in a constant direction. It suggests that some climatic condition favoring or opposing the development of fruits may cause definite variation in the form of fruits, although this apparently has no connection with their absolute size.

Data obtained from a study of the relation between the variation in the form of fruits and the climatic conditions prevailing during the growing season for each of three years are available. The average

TABLE V

AVERAGE MONTHLY TEMPERATURE (°C.) DURING GROWING SEASON

Year	April	May	June	July	August
1918.....	12.8	16.9	21.1	26.7	26.6
1919.....	12.6	15.4	19.4	23.5	24.8
1920.....	12.7	16.9	20.5	25.9	25.1

TABLE VI

TOTAL MONTHLY PRECIPITATION (MM.) DURING GROWING SEASON

Year	April	May	June	July	August
1918.....	166.4	131.7	128.7	55.0	154.9
1919.....	78.1	58.4	144.2	206.4	154.4
1920.....	173.7	285.9	252.2	241.2	522.8

temperature and the total precipitation from April to August are shown in tables V and VI. The average temperature for three years during each of the three weeks following full bloom is shown in table VII.

The Chojuro pear usually commences to blossom from the first to the fifteenth of April, and is harvested in late August or early September. The growing season of the fruit, therefore, in that part of Japan where the experiments were conducted, is not longer than five months. Table VI shows that the average temperature for each of the five months was lowest in 1919 and highest in 1918. The average value of the form index for each plot, without exception, was smallest in 1919 and largest in 1918. The annual difference in aver-

age temperature for each month was more noticeable in June, July, and August than in April and May. It is reasonable to suppose that there is a close correlation between the form of fruits and the average temperature during the growing season. No relationship was found between the monthly precipitation and the variation in the form of fruits.

Nothing of marked interest is indicated by a comparison of the average temperature during the three years for each of the three weeks following full bloom. In 1918 blooming continued from April 12 to 30, and the weekly temperatures were observed from the middle of the blooming date, or April 21. In 1919 the trees were in flower from April 3 to 15, and the observations were begun on the

TABLE VII

AVERAGE TEMPERATURE (°C.) FOR THREE WEEKS FOLLOWING FULL BLOOM

Year	First week	Second week	Third week
1918.....	13.7	14.7	15.9
1919.....	12.5	14.5	13.1
1920.....	11.8	14.4	18.1

ninth. In 1920 the flowering continued from April 15 to 27, and the observations were started on April 21. From results presented in table VII it appears that there is little or no correlation between the form of fruits and the average temperature of the three weeks immediately following full bloom.

The time of maturity of the fruits in the different plots and the length of the growing season were noted. In 1918 the fruits in each plot matured earlier than in the other two years. All fruits were harvested on August 24 and 25. Their growing season was 126 days. The fruits produced in the check plot showed reddish brown color on August 15 or 16, and four or five days later those in the *K* plot began to mature. The *NP* and *N* plots matured last. There was little difference in the ripening dates of the other four plots. In 1919 all fruits were harvested on September 3 and 4. The growing season this year was 150 days. The fruits in the *K* plot were the earliest in their maturity, and those in the *NP* and *N* plots the latest. The ripening date of the check plot corresponded closely with that of the

K plot, but the amount of fruit harvested from the check plot was so small as to be of no value for comparison with other plots. In 1920 all fruits were picked on September 5 and 6. Their growing season was 138 days. The *K* plot showed the earliest, and the *NP* and *N* plots the latest maturity, as in the previous years. The trees in the check plot began to grow vigorously this year, which may account for the delayed maturity.

These results indicate that the form of fruits is influenced by the average temperature during the growing season, which hastens or delays the maturity of the fruits. It is also probable that variation in the form of fruits depends somewhat on the treatment of the tree, and that this treatment also affects the length of the growing season. The earlier the maturity the more flattened is the form of fruits.

Effect of growing season upon size and form of fruit

In the previous discussion a single tree or a certain plot was taken as a unit for the variation in the size and form of pears. The various stages of individual fruits, however, represent a life history of development. It is important, therefore, to observe the life history of the individual specimen in the study of variations in size and form.

Studies were made to determine whether there is a more or less definite period when pears make their chief increases in bulk and attain their fixed form, or whether increases in size and variation in form are both quite uniform and continuous throughout the growing season.

RIVIERE and BAILHACHE (1) found, in the case of pears, that there are more or less definite growth periods for the developing fruit. Winter and many fall varieties increase in size just previous to the time for picking, while summer and a few fall varieties make their chief increase in size about the middle of their growth.

WHITEHOUSE (6) reported, in his study of variation in apples during the growing season, that at least in the case of the Grimes there was a comparatively steady increase in size from the time of setting of the fruit until full maturity. There was no period of especially rapid growth, as reported by RIVIERE and BAILHACHE for pears. There was no especially marked increase in size of the Grimes

apple after the first of the fall rains in early September. WHITEHOUSE therefore stated that one would hardly expect to find such increases following a rain or irrigation during the growing season. He also reported in the case of the Tompkins King and the Fameuse varieties that the apples remained fairly constant in form, as compared with each other, throughout the growing season. In the Fameuse variety there was a tendency for apples to increase in transverse diameter relatively more rapidly than in longitudinal diameter; in other words, they became more oblate as the season progressed. This tendency was most pronounced in the early part of the growing season.

TAYLOR and DOWNING (**5**), in their studies on the irrigation of apple orchards, reported that Jonathan apples grew slowly from the time they were formed until July 15, completing less than 30 per cent of their total growth during the first half of the season. Irrigation during this period (before July 15) did not increase the size of the apples. Jonathan apples grew most rapidly during the period beginning about July 15 and ending about two weeks before picking time, when the rate of growth became considerably slower. Irrigation during this period of rapid growth had a decided effect in increasing the size of the apples, although it had almost no effect on the wood growth of the tree.

The writer's studies were carried on in 1918, 1919, and 1920. The first two years the observations were made on fruits on a tree grown in a plot treated with complete fertilizers, which was separated from the experimental fertilizer plot. The third year, trees no. 5 of both the *NPK* and *NP* plots were chosen for observation. The index for the form was obtained as previously outlined. The index for showing the size was deduced from the product of the transverse and longitudinal diameters of the fruit. The correlation between the real weight of the fruit and this index was studied. The coefficient of correlation was $+0.9188 \pm 0.0038$, calculated from 1616 matured fruits in 1918. From this result, the figure derived from the product of both diameter and length could be used with some confidence as a relative index to determine the size or weight of fruits, although the specific gravity of fruits might be different in the various stages of

growth. The intervals between measurements were from seven to eleven days, the irregularity being necessitated by weather conditions and other circumstances.

Originally 25 pears were selected for observation in 1918, and 24 of them were measured regularly. The other fruit dropped prematurely. In 1919, 40 pears were observed at the start, 30 of which were measured throughout the growing season. In 1920, 35 pears were originally selected on the tree in each of the two plots, but only 20 on tree no. 5 of the *NPK* plot and 23 on tree no. 5 of the *NP* plot were measured regularly.

Records were kept of the average temperature and the total precipation throughout the growing season, so that it was possible to correlate these factors with any marked variation in rate of fruit growth or in the proportion in growth of both diameter and length. These data, together with the average indices of both the size and the form of the fruits in each period of measurement, are shown in tables VIII–XI.

Tables VIII and IX show that there was a period of especially rapid growth, comparable with that found for pears by RIVIERE and BAILHACHE. The fruit rapidly increased in size during this period as compared with other periods. It appeared in the interval between July 8 and 18 in 1918, and from June 28 to July 7 in 1919. In 1918 the maximum rate of growth took place during the period starting July 28 and ending August 6, or from the 99th to the 109th day following the date of full bloom. In 1919 the maximum rate of growth was during the period from July 18 to 28, or the interval between the 101st and the 112th day from full bloom.

The writer has applied the name "maximum period" to the period in the growing season during which the maximum rate of growth per day took place. When the fruit passed the maximum period the rate of increase in size became gradually slower until the harvesting time, in both 1918 and 1919. It is to be noted in both tables that there was not an especially marked increase in size following a period of rain.

With variation in form, the index increased continuously as the season progressed to the maximum period, or the period a little after it. The writer's studies gave much the same results as those obtained

TABLE VIII

AVERAGE INDICES OF SIZE AND FORM IN EACH PERIOD OF MEASUREMENT; DAILY INCREASE IN SIZE AND INCREASE OR DECREASE OF FORM INDEX PER PERIOD, INCLUDING METEOROLOGICAL DATA (1918)

Data observed	May 28	May 28–June 7	June 8–18	June 19–29	June 30–July 7	July 8–18	July 19–29	July 28–August 6	August 7–16	August 17–24
Size in last of each period	475.79	640.07	887.93	1125.57	1375.29	1955.79	2509.14	3204.14	3844.50	4203.79
Increased rate in size per day	.....	14.92	22.53	21.60	31.22	52.77	61.48	69.50	64.04	44.91
Form in last of each period	112.93	115.57	116.00	117.86	120.86	122.71	124.36	124.64	122.29	122.71
Variation in form in last of each period	.....	+2.64	+0.43	+1.86	+3.00	1.85	+1.65	+0.28	−2.35	+0.42
Average temperature (°C.)	.....	19.6	20.3	22.6	27.0	25.6	27.3	27.3	26.8	27.1
Total precipitation (mm.)	.....	24.5	30.9	82.9	14.6	40.4	.....	34.2	37.3	0.0

TABLE IX

(1919)

Data observed	May 15–27	May 28–June 7	June 8–17	June 18–27	June 28–July 7	July 8–17	July 18–28	July 29–August 6	August 7–16	August 17–26
Size in last of each period*	612.77	839.83	1035.27	1321.17	1832.30	2538.43	3424.93	4132.17	4791.70	5309.50
Increased rate in size per day	17.07	20.64	19.54	28.59	51.11	70.61	80.59	78.58	65.95	51.78
Form in last of each period†	109.30	111.90	114.47	117.07	119.07	119.13	117.13	117.43	117.53	117.77
Variation in form in last of each period	+4.43	+2.60	+2.57	+3.20	+1.40	+0.06	−2.00	+0.30	+0.10	+0.24
Average temperature (°C.)	14.5	18.5	18.0	21.1	22.4	22.6	24.8	24.9	24.3	23.7
Total precipitation (mm.)	10.8	76.3	39.1	28.2	31.3	12.6	8.2	210.5	97.7	1.3

* 390.80 on May 14.

† 104.87 on May 14.

by WHITEHOUSE in his studies on apples. When the fruit passed the maximum period some irregularities took place in the direction of variation in form. These results indicate that the maximum period is the most important time for the observations on the variation in the pear during its growing season.

A comparison of the data presented in tables X and XI is of interest. The observations were made after June 23 in 1920. The fruits on tree no. 5 of the *NP* plot averaged 87 per cent as large as those on tree no. 5 of the *NPK* plot at the beginning of the measurements. At harvesting time on September 6, the former was 41 per cent the size of the latter. The maximum period was reached one period earlier in the *NP* than in the *NPK* plot. In the former case the maximum period appeared in the interval between the 83d and the 92d day from the date of full bloom. In the *NPK* plot the period of most marked increase of the daily rate of growth coincided with the maximum period. There was no period of rapid growth in the *NP* plot, but the rate of growth became low after early August. The rate of growth was also slow in the season previous to the maximum period in the *NP* as compared with the *NPK* plot. With regard to variation in form, the same results were obtained as in 1918 and 1919. The data presented in the four preceding tables, although showing some irregularities, indicate that the higher the temperature the more flattened the fruits, especially in the weeks previous to the maximum period.

Summary and conclusions

1. There is no noticeable correlation between the form and the size of fruits produced on an individual tree of the Chojuro variety of *Pyrus serotina*.

2. There is a close relationship between the form of fruits and the length of the growing season.

3. The form of fruits is influenced by the average temperature during the growing season, which hastens or delays their maturity.

4. No relationship, however, is observed between the monthly precipitation and the variation in the form of fruits.

5. It is probable that variation in form of fruits depends somewhat on the treatment which affects the length of growing season or ripening period. The earlier the maturity the more flattened is the form.

TABLE X

NPK PLOT (1920)

Data observed	June 23	June 24–July 1	July 2–11	July 12–21	July 22–August 1	August 2–11	August 12–16	August 17–26	August 27–September 6
Size in last of each period	859.20	1013.05	1338.25	1833.95	2605.20	3230.30	3427.80	3813.65	4141.70
Increased rate in size per day	.....	19.23	32.52	49.57	70.11	62.51	39.50	38.59	29.82
Form in last of each period	108.90	109.85	112.95	116.00	117.70	118.55	119.95	119.85	119.15
Variation in form in last of each period	.....	+0.95	+3.10	+3.05	+1.70	+0.85	+1.40	−0.10	−0.70
Average temperature (° C.)	.....	21.3	25.9	26.3	25.9	24.6	25.7	25.5	24.5
Total precipitation (mm.)	.....	29.4	35.8	0.2	205.2	344.3	26.4	147.4	139.6

TABLE XI

NP PLOT (1920)

Data observed	June 23	June 24–July 1	July 2–11	July 12–21	July 22–August 1	August 2–11	August 12–16	August 17–26	August 27–September 6
Size in last of each period	758.00	936.52	1185.00	1466.00	1737.26	1897.91	1934.43	1994.96	2061.22
Increased rate in size per day	.....	22.32	24.85	28.10	24.66	16.07	7.30	6.05	6.02
Form in last of each period	112.22	113.26	117.52	119.17	120.70	119.78	119.91	120.26	118.65
Variation in form in last of each period	.....	+1.04	+4.26	+1.65	+1.53	−0.92	+0.13	+0.35	−1.61
Average temperature (° C.)	.....	21.3	25.9	26.3	25.9	24.6	25.7	25.5	24.5
Total precipitation (mm.)	.....	29.4	35.8	2.0	205.2	344.3	26.4	147.4	139.6

6. The term "maximum period" has been applied to the period in the growing season during which the maximum rate of growth per day takes place.

7. The maximum period of Chojuro pears, in the Kanagawa Agricultural Experiment Station plots, appears about the 100th day from the date of full bloom, when the fruit develops under normal conditions.

8. Generally the form of the pear becomes more and more oblate in the season previous to the maximum period, and there is no definite direction of variation in the form in the season following this maximum period.

9. The writer does not consider the data conclusive; nevertheless they indicate that when relatively high temperatures prevail during the growing season previous to the maximum period, the form of fruits is more flattened than when lower temperatures exist.

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CONDITIONS FOR GERMINATION OF SPORES OF ONOCLEA SENSIBILIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 336

CONSTANCE E. HARTT

(WITH PLATE XXIX, AND ONE FIGURE)

Conflicting accounts have been given by various workers concerning the conditions for germination of fern spores. No adequate explanations have been made of these discrepancies. As the results of each investigator are usually consistent, it seems probable that some undescribed conditions of experimentation vitally affect the work. The factors which have been noted as influencing germination are temperature, intensity and refrangibility of light, culture media, and age of spores, and it is to be regretted that these data are often insufficient in the literature.

Historical

No mention is made of temperature by KAULFUSS, LESZCYC-SUMINSKI, THURET, MERCKLIN, WIGAND, or HOFMEISTER, except that MERCKLIN considered a certain temperature necessary. BORODIN (**3**), using water cultures of *Aspidium spinulosum*, found that the spores exposed to full lamplight germinated before those exposed to lamplight from which the heat rays were absorbed by interposing a vessel filled with water. SCHELTING (**17**) investigated the influence of temperature on germination, and found that *Ancimia Phyllitidis* always germinated earlier at 30° C. than at 18° C., and with further development showed that at the higher temperature the cells divide more rapidly but grow in length more slowly. He further stated that higher temperatures will cause germination in darkness, a statement which WORONEW contradicted. WORONEW (**20**) obtained germination in the dark on a nutrient solution at 28° C., but claimed that germination was slower than at a lower temperature in the light.

FISCHER (8), however, stated that spores of *Polypodium vulgare* germinated better in the dark at 25° C. than in daylight at room temperature, but no mention is made of the culture medium used. According to SCHULTZ (18), other stimuli are not able to replace light, except in the case of *Ceratopteris thalictroides*, in which high temperature may. HEALD (9), working with *Alsophila Loddigesii* and *C. thalictroides*, found that a temperature of 32° C. will furnish the necessary stimulus for germination in the dark. He stated that germination depends upon certain chemical changes in foodstuffs stored in the spores; these changes may be caused by light or a temperature of 32° C. KNY (12), working with *C. thalictroides*, found that favorable temperature conditions are necessary, but did not state the temperature specifically.

Intensity of light is mentioned by several investigators. LIFE (14) found that germination is best in light of medium intensity. BECK (2) and KNY (11) both found that light of "sufficient intensity" is a necessary condition for germination. According to NAGAI (15), strong light is injurious to the germination of spores of *Ceratopteris thalictroides*, and according to WORONEW bright light retards growth. The effect of weak light was discussed by HOFMEISTER (10): "In those species where considerable elongation of the lowest cell occasionally takes place, it seems to be caused by deficiency in light."

Considerable work has been done to ascertain whether or not spores will germinate in absolute darkness, the varied results of which are given in table I.

SCHELTING found that spores germinate well in the dark, and form a small amount of chlorophyll which is lacking in the spore, but LAAGE claimed that chlorophyll is not formed during germination in the dark. He obtained no stages in germination in the dark more advanced than the bursting of the exine.

Results on the effect of different qualities of light are given by BORODIN, SCHULZ, HEALD, and BURGERSTEIN (5). BORODIN, having found to his satisfaction that light is a condition necessary for germination, proceeded to determine which light rays are the most effective. Using water cultures of *Aspidium spinulosum*, *Aneimia Phyllitidis*, and *Allosorus sagittatus*, he was able to germinate spores in yellow light but not in blue. He isolated yellow rays by passing

TABLE I

GERMINATION IN THE DARK

Investigator	Fern	Result	Temperature (C.)	Medium
Borodin (1868)	Aspidium spinulosum var. foeniscii	o		Neva River water
	Aspidium molle	o		" " "
	Aneimia Phyllitidis var. longifolia	o		" " "
	Allosorus sagittatus	o		" " "
	Asplenium alatum	o		" " "
	A. (Displasium) lasiopteris	o		" " "
	Polypodium repens	o		" " "
	Phegopteris effusa	o		" " "
Goeppert (1869)	Osmunda sp.	+		
Schmidt (1870)	Aspidium Filix-mas	o		
	Aspidium violaceus	o		
Kny (1872)	Osmunda sp.	o		
Schelting (1875)	Aspidium Filix-mas	+		Soil and water
	Aspidium falcatum	+		" " "
	Aneimia Phyllitidis var. longifolia	+	30° and 18°	
	Pteris aquilina	+		" " "
Beck (1878)	Scolopendrium vulgare	o		
Heald	Alsophila Loddigesii	o	19–21°	Filter paper, pieces of flower pots, sterile earth in Petri dishes
	Ceratopteris thalictroides	o	19–21°	
	Ceratopteris thalictroides	+	32°	
Burgerstein (1900)	Osmunda sp.	o		Haide erde and peat
	Pteris (3 spp.)	o		" " " "
Schulz (1902)	Aspidium falcatum	o		
	Aspidium Sieboldii	o		
	Asplenium Filix-foemina	o		
	Polypodium aureum	o	Ordinary	
	Didymochlaena lunulata	o	tempera-	
	Doodia caudata	o	ture	
	Scolopendrium officinale	o	and	
	Gymnogramme chrysophylla	o	35°	
	Aneimia Phyllitidis	o		
	Alsophila elegans	o		
	Alsophila australis	o		
	Ceratopteris thalictroides	+	High	
Laage (1906)	Osmunda regalis	+	Room	Distilled water
	Pteris aquilina	+		Knop's solution
	Scolopendrium officinarum	+		" "
	Aspidium Filix-mas	+		" "
	Polypodium Dryopteris	+		" "
	Pteris cretica	+		" "
	Aspidium aculeatum	+	19°–21°	" "
	Aspidium spinulosum	+	and	" "
	Balantium antarcticum	+	30°	" "
	Asplenium lucidum	o		" "
	Alsophila australis	o		" "
	Polypodium aureum	o		" "
Life (1907)	Dicksonia apiifolia	o		
	Alsophila pruinata	o	30°–33°	Leaf mold
	Aneimia Phyllitidis	o	30°–33°	" "
	Gymnogramme calomelanos Mertensii	+	30°–33°	0.5 per cent Knop's solution
	Gymnogramme calomelanos Mertensii	o	Room	Leaf mold
Nagai (1914)	Adiantum tenerum	o	15°, 25°–27°	0.5 per cent Knop's solution in agar-agar
	Adiantum fulvum	o	"	" "
	Adiantum peruvianum	o	"	" "
	Adiantum macrophyllum	o	"	" "
	Asplenium bulbiferum	o	"	" "
	Asplenium Belangerii	o	"	" "
	Woodwardia radicans	o	"	" "
	Nephrolepis davallioides	o	"	" "
	Nephrolepis exaltata	o	"	" "
	Cibotium Schiedei	o	"	" "
	Balantium antarcticum	o	"	" "
	Ceratopteris thalictroides	+	"	" "
	Aspidium Filix-mas	o		Organic and inorganic nutrient solution
	Aspidium fulvum			

light through a solution of potassium dichromate; for blue rays, he used ammoniated copper oxide. He concluded that germination is due to the less refrangible rays, those of higher refrangibility acting in a manner similar to complete darkness, or in any case their effect being much weaker than the yellow rays. SCHULZ found that moss and fern spores germinate in the yellow and blue halves of the spectrum, as he obtained germination of several species of fern spores in white and red rays in twenty days, and in blue in thirty days. HEALD, working with *Marchantia* spores, found that they do not germinate in darkness, and that blue rays have the same effect as absolute darkness. BURGERSTEIN, using twenty-four species of ferns, found that under the influence of blue rays the prothallia were formed several days to weeks later, never earlier, than with the less refrangible rays. No prothallia of *Gymnogramme sulfurea* were formed in blue light.

Data on the age of spores show varied results. TREBOUX (**19**) stated that old moss spores often show slight viability in darkness, and will germinate in light only when provided with glucose. Miss ROGERS (**16**), working with *Lygodium palmatum*, found that spores planted at the end of three months produced less vigorous prothallia than those planted directly after collection. She found that each successive planting produced prothallia of decreasing vigor, as shown by their smaller size and slower growth. The rate and percentage of germination were decreased. Practically all the spores planted directly after collection germinated, but of those which were kept seven months, 50 per cent began to crack only after twelve days. CAMPBELL (**6**) found that the spores of *Onoclea Struthiopteris* contain considerable chlorophyll, and germinate in three to five days. They retain their viability several months after being gathered.

Several examples of the longevity of fern spores are found in the literature. COLEBY and DRUERY (**7**) reported the germination of spores of *Dicksonia* twenty years old, and of spores of *Gymnogramme aurea* which must have been dormant a long time. According to WRIGHT (**21**), spores of *Cheilanthes mysurensis*, which were collected in October, 1899, and preserved in a herbarium, were sown in March, 1908, and produced healthy plants. In 1910 (**1**) a report appeared of

spores of *Dicksonia antarctica* which produced a large number of healthy plants after being in a herbarium twenty-two years. LAAGE, however, was unable to germinate six-year old spores of *Aspidium Filix-mas*, *Aspidium angulare*, *Alsophila procera*, or *Dicksonia Wendlandi*.

According to KNY (11), old spores of *Osmunda regalis* show an appearance deviating very much from that of fresh spores. Their content is shrunk. The oily drops have come together in one or more larger drops. Because of thinness of exine and rich chlorophyll content, their germination capability is of short duration.

While these investigators have shown that age is a factor in decreasing the germination capability of spores, LIFE has shown, in one case at least, that age is important in causing germination. *Alsophila australis* spores need a year's rest period between collection and germination.

Materials and methods

It is apparent that the germination of spores should be studied from the point of view of limiting factors. The experiments recorded here deal with the following factors: intensity of light, effectiveness of rays of different refrangibility, continuous light, temperature, and age of spore. The culture medium in all cases was distilled water. One species of fern was used, *Onoclea sensibilis*. In all experiments efforts were made to keep all conditions uniform except one factor.

Varying intensities of light were obtained under the following conditions: (1) full sunlight, all day; (2) intense sunlight, south window with sunlight for six hours in middle of day; (3) moderate sunlight, east window with sunlight four hours in morning and diffused light rest of day; (4) intense diffused light, north window; (5) moderate diffused light, laboratory table 18 feet from north window; (6) weak diffused light; (7) absolute darkness, as indicated by photographic tests. Constant temperature was maintained in water baths with thermostat control.

Rays of different refrangibility were obtained by using red and blue colored glass prepared at the Desert Botanical Laboratory at Tucson, Arizona. The red glass admitted only red rays of the spectrum, while the blue admitted green, blue, and violet rays, when

tested with a spectroscope. Cultures were kept under red and blue glass in north, east, and south windows, and in continuous light. Continuous light of constant intensity was obtained by using an electric bulb in a light-proof, wooden box lined with white paper.

To ascertain the relation between viability and age of spores, experiments were made with fresh material collected when shedding, and some which had been stored in a refrigerator at 12°–13° C., and some stored at room temperature for periods varying from four weeks to ten months. The spores of *Onoclea sensibilis* are formed during the summer and fall, and remain over winter in the sporangia in apparently mature condition. They are shed in the open, in Massachusetts, during April and the first part of May. These large, oval spores vary in size from 58 to 73 $\mu \times 67$ to 76 μ, and contain considerable chlorophyll. The spore of *O. sensibilis* is similar to that of *O. Struthiopteris*, as described by CAMPBELL, in having three thin spore coats through which the single large nucleus may easily be seen. KNY also found that the nucleus of the spores of *Osmunda regalis* is often visible in an uninjured spore. When this is not possible, a slight pressure on the cover slip will cause the spore to emerge from its outer coat, thus facilitating observation of the earlier stages of germination which take place before the rupture of the exine. The first step is the division of the nucleus. The resulting two daughter nuclei may frequently be seen without the removal of the exine, before the dividing wall has formed. The splitting of the exine normally takes place after the formation of the first wall. The succeeding stages are of the usual polypod type (figs. 1–7). Germination in favorable conditions in the laboratory requires two to three days, but has been obtained in twenty-six hours. In April, 1922, the germination period in the open, when the temperature varied 5°–16° C., was one week, with a very low percentage of germination.

Observations and results

EFFECT OF DIFFERENT LIGHT INTENSITIES

As these experiments, lasting from February 10 to April 18, gave consistent results, an account of a typical set will be given. On April 10, spores were planted on distilled water within two to four

hours of collection. The temperature ranged from 21° to 23° C. The spores receiving intense diffused light always germinated most rapidly and in greatest numbers. Those receiving moderate and weak diffused light had a longer germination period than those in intense diffused light, while those in absolute darkness germinated even more slowly. In general, those in absolute darkness and those in weak diffused light developed longer filaments than those receiving intense diffused light, as a result of which the chlorophyll of the former was less dense (figs. 8–10), but some germinated normally. Those in moderate sunlight germinated more slowly and less abundantly. Very few germinated in intense sunlight, many losing their

TABLE II

RELATIVE STANDING AFTER 29–34 HOURS

1. Full sunlight	0
2. Intense sunlight	1 out of many
3. Moderate sunlight	13 out of 100
4. Intense diffused light (in other experiments a larger proportion germinated)	24 out of 52
5. Moderate diffused light	21 out of 59
6. Weak diffused light	10 out of 36
7. Absolute darkness	6 out of 30

chlorophyll (fig. 11). There was no germination in full sunlight, most of the spores losing their chlorophyll and dying. The results of this experiment are shown in table II and text fig. 1.

The question of chlorophyll formation in the dark is a difficult one. Resting spores contain chlorophyll but in no definite shape, while filaments produced in the light or dark contain chloroplasts; therefore some change takes place, the nature of which was not studied.

EFFECT OF TEMPERATURE

Data on the effect of temperature on germination were obtained in intense diffused light, absolute darkness, and continuous light. The experiments with continuous light are discussed later.

The object of these experiments was to determine the minimum, optimum, and maximum temperatures at which germination takes place in intense diffused light and in absolute darkness. Experiments

conducted by Miss STOKEY of Mount Holyoke College, assisted by a student, and others by the writer, lead to the supposition that the minimum temperature for germination in intense diffused light is around 15° C., the optimum between 27° and 33°, and the maximum between 35° and 40°. These experiments were conducted without absolute temperature regulation.

Using electrically controlled water baths, a series of experiments was conducted in intense diffused light and absolute darkness, with the results as shown in table III.

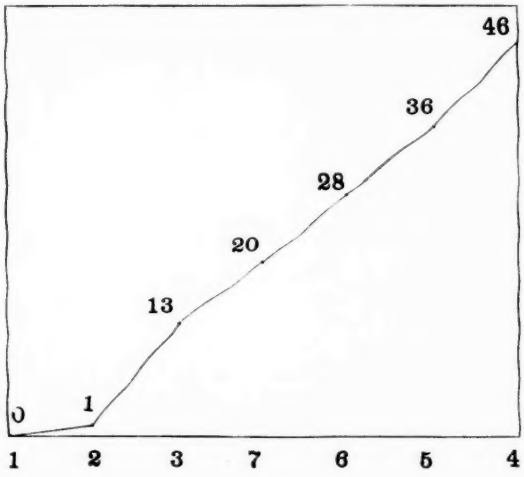

FIG. 1

That 23 per cent germination was obtained at 17° C. seems not to be in accord with the results at 16° and 18°. It is possible that a slight variation in the temperature occurred during the night. The table shows that germination was first obtained at 16° in the light, and about 24° in the dark; that the highest percentage of germination in the light was obtained at 28°, and in the dark at 28°–29°; that the maximum temperature for germination was 34° in the light and 33° in the dark.

Not only does temperature affect the percentage of germination, but also the character. Prothallia produced at 16°–28° in the light

were similar as to size of cells and distribution of chlorophyll. Those at 28° were further advanced in three days than those at the lower temperatures. Those at 29°–31° showed abnormal growth; for example, twinning, bent prothallia, rounded cells, and chlorophyll concentrated in the tip cell (figs. 12, 13). Some ungerminated spores at 31°–34° contained large fatty globules, resembling old spores. Spores kept at 35°–40° showed diminution in amount of chlorophyll.

TABLE III

GERMINATION IN THREE DAYS

Temperature °C.	Light	Dark
12	0	0
13	0	0
14	0	0
15	0	0
16	2 (10 per cent in 4 days)	0
17	23 per cent	0
18	14 per cent	0 (1 doubtful)
21	20 per cent	0
24	Many	1
27	Many	6–7 per cent
28	100 per cent (normal)	95 per cent
29	85–90 per cent (abnormal)	95 per cent
30	90 per cent (abnormal)	30 per cent
31	30 per cent	30 per cent
32	Abnormal	16–20 per cent
33	Very few	Very few
34	Very few	0
35	0	0
36	0	0
40	0	0

Spores were kept at 35° for three days and then at room temperature. In three days a few had germinated, showing that they were not killed by 35° C. Spores which had been kept at 36° for three days were placed at room temperature and four germinated in two days; 40° was found to kill the spores.

It is probable that germination depends upon the activity of certain enzymes. Possibly their reactions toward temperature may be explained by certain facts true of other physiological reactions; namely, that at normal temperature their actions are coordinated, but just below the maximum temperature there is more or less loss of self-regulation and coordination, which stops growth without

killing the organism. If the unfavorable condition does not last too long, activities become normal again with returning normal conditions; but if the temperature rises too high, cell death occurs.

Effect of rays of different refrangibility

The spores kept under red and blue glass in intense sunlight did not germinate. As with full light, these experiments showed that intense diffused light is more favorable for germination than partial direct sunlight. In twenty-nine hours, half of those receiving the red rays in intense diffused light were beginning to germinate, whereas only one-fifth of those receiving the more refrangible rays were germinating. This experiment was repeated several times, and every time those under the red glass in intense diffused light germinated the most successfully. These results, therefore, agree with those of Borodin and Heald, in that the less refrangible rays of the spectrum were found to be the most effective in promoting germination. The results differ in that the writer obtained germination with the more refrangible rays, while they did not. They experimented with spores containing little or no chlorophyll, however, while those of *Onoclea sensibilis* contain a large amount.

Effect of continuous artificial light

Almost all the spores kept at 31° and receiving full light germinated in forty-two hours, the exine splitting and green protruding. The primary rhizoid of some spores had begun to develop. A very few of those in the dark were cracking, many showing partial or complete loss of chlorophyll. Almost all germinated in red light, while only half germinated in blue. Two of those at 37° receiving full light germinated; the others were less green than normal, the chlorophyll having shrunk to the centers of the spores. Of about two hundred in the dark, nine or ten germinated. All the spores were greener than those in the light, but not as green as normal. A few of those receiving red rays germinated, but were not normally green. Those receiving blue rays showed results similar to those in absolute darkness. The temperatures mentioned are doubtful, as there was no adequate means of control. Subsequent work with more accurate control makes it seem highly improbable that any germination took place at 37° C.

Viability of spores of different ages

These experiments were undertaken because the best results obtained in absolute darkness were in February and March, while in the last part of April and the first part of May the percentage of germination was low, and in the last part of May there was no germination at all. The spores of the first experiments were used a few hours after collection, whereas those used later had been stored four to six weeks at 12°–13°. It would appear from this that fresh material is the best. To prove this, an attempt was made in the last part of May to find fresh material in the open. After a successful search it was found that material collected in the last part of May will not germinate in darkness in the usual time. The majority germinated in eight days, a few with long filaments of only one cell, but the greater number with two normal cells. These results suggest that germination is influenced by the age of spores.

Spores were collected near South Hadley, Massachusetts, in May, 1923, and stored about a month at 12°–13°; afterwards at room temperature. At the end of March, 1924, many of these germinated in light in five days, and a very few in the dark in ten days.

Spores were collected near Smith, Indiana, July 7, 1923, and stored at room temperature. From January 4 to 10, 1924, a few germinated in light and in the dark, but from March 28 to April 7 there was no germination either in light or dark, and there was no visible chlorophyll in the spores. Spores were sown May 8 and kept in intense diffused light at 28°–30°. By May 13 five spores had germinated, while most of the others were brown or almost colorless. It was evident from the position of these five spores that they had come from a single sporangium which had afforded them protection.

Spores collected December 17, 1923, at Greensboro, North Carolina, and stored at room temperature, were planted March 28. After five days in the light many were two or three celled, while after ten days in the dark only three germinated, forming long, single celled prothallia with the chlorophyll in the tip. These results suggest that spores retain their germination ability in light longer than in the dark.

Little work has been done to determine the chemical difference

between mature and old spores. Fresh spores contain fat (but not in conspicuous globules), a little protein, and no starch. They are richly supplied with chlorophyll. The older a spore becomes the less chlorophyll it contains, until none is left. Old spores contain many conspicuous fatty droplets of various sizes (fig. 14). Similar globules may be obtained by heating fresh spores in 95 per cent alcohol on the water bath.

While these factors have been treated separately, it must not be supposed that they can be absolutely divorced. For instance, old spores will germinate at higher temperatures but not at medium or low, and in intense diffused light spores will germinate at temperatures lower than in absolute darkness. Since *Onoclea sensibilis* spores germinate over a wide range of light and temperature intensities, but lose their viability in a comparatively short time, it is suggested that age may be the limiting factor.

Summary

1. These experiments deal with the conditions for germination of spores of *Onoclea sensibilis*.

2. Spores germinate best in diffused light, and intense diffused light is better than moderate or weak.

3. Sunlight is unfavorable to germination, and intense, long continued sunlight destroys chlorophyll.

4. Spores germinate in absolute darkness.

5. The highest percentage of germination in continuous light was obtained at 31° C.

6. The minimum temperature for germination in intense diffused light is 16°, the optimum 28°, and the maximum 34°; 40° kills the spores.

7. The minimum temperature for germination in absolute darkness is 24°, the optimum 28°–29°, and the maximum 33°.

8. The less refrangible rays of the spectrum are the most effective in causing germination, whether intermittent or continuous.

9. Viability in light and darkness depends upon age of spores. Spores retain the ability to germinate in light longer than the ability to germinate in the dark.

10. Old spores differ from fresh ones in that they contain less chlorophyll, and the fat is present in more conspicuous globules.

11. Since *O. sensibilis* spores germinate over a wide range of light and temperature intensities, but lose their viability in a comparatively short time, it is suggested that age may be the limiting factor.

12. It is suggested that the conflicting accounts of previous investigators have been caused by some undescribed conditions of experimentation, such as variations in temperature, light intensity, types of media, or age of spores.

The writer wishes to express deep gratitude to Dr. ALMA G. STOKEY of Mount Holyoke College, and to Dr. CHARLES A. SHULL of the University of Chicago, for suggestions, help, and criticism of these experiments.

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EXPLANATION OF PLATE XXIX

All figures except fig. 14 were drawn with a camera lucida×340.

FIGS. 1–7.—Stages of normal germination.

FIGS. 8–14.—Abnormal forms.

FIG. 8.—After 72 hours in weak diffused light.

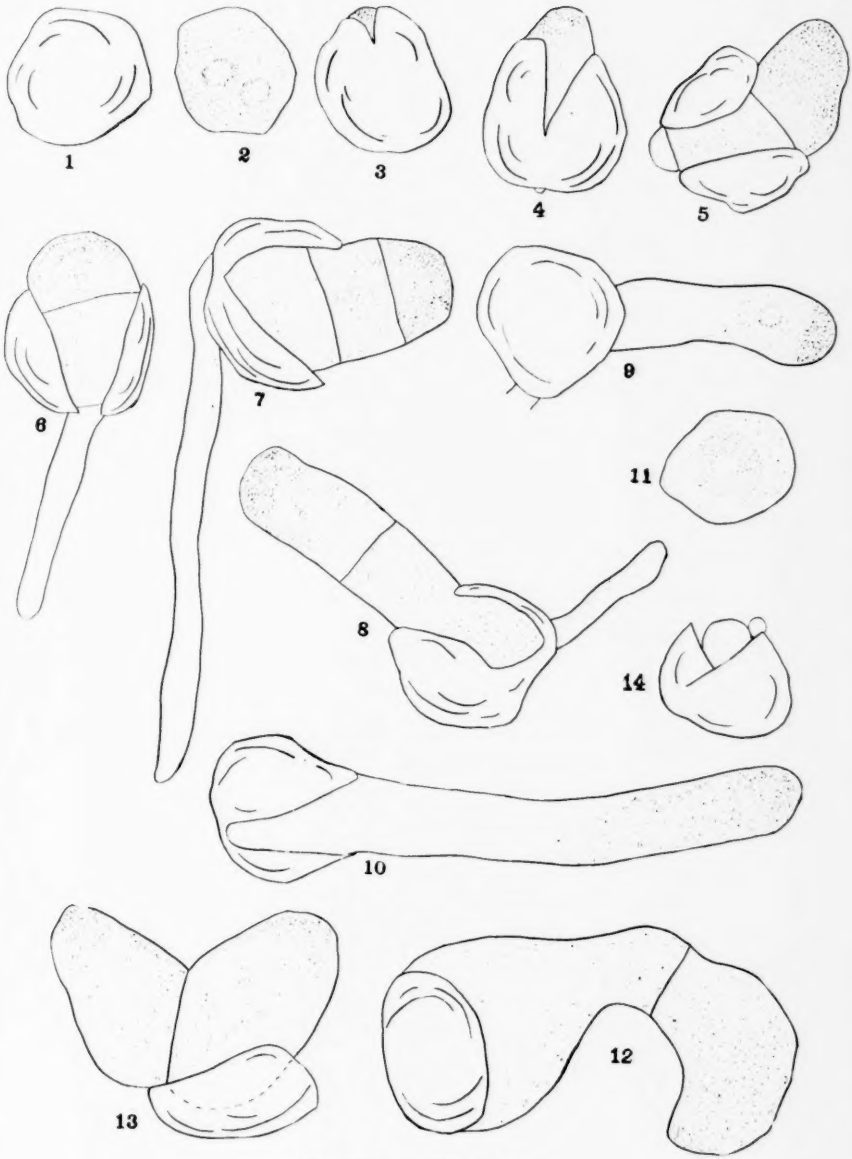
FIG. 9.—After 66 hours in absolute darkness.

FIG. 10.—After 72 hours in absolute darkness.

FIG. 11.—Diminution of chlorophyll content, as in old spores, or those exposed to intense sunlight or to temperatures of 31°–40° C.

FIGS. 12, 13.—Bent prothallium and twins formed at 29°–31° C.

FIG. 14.—Crushed spore with fatty globules.



HARTT on ONOCLEA

A NEW SPECIMEN OF LEPIDOSTROBUS FOLIACEUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LARORATORY **337**

JUAN D. CAMPOS

(WITH PLATES XXX–XXXII)

The series of sections which forms the principal basis of this paper was sent by Mr. LOMAX of Lancashire, England. As the type specimen (C. N. 1614, WILLIAMSON **8**) on which MASLEN founded this species was incompletely preserved, only a provisional diagnosis (**4**) was given. With better material on hand, excellently preserved and prepared, it seemed wise to direct attention to an intensive study of the structure of this species so that a fuller description of it could be given.

The material studied consists of a series of twelve transverse sections of the cone, slides G 8–19. According to LOMAX, it is "the most perfect specimen of a lycopodiaceous cone that has yet been discovered." A comparison of this with those described and figured in literature will justify the claim. G 8 is cut through the apex and G 19 through the base of the cone. The length of the cone was originally 2 inches, or 5.1 cm., while measurements of the middle sections give an average of 2.4 cm. in diameter. The Halifax Hard Bed at Huddersfield, Yorkshire, England, from which the specimens were collected, is a coal seam of the Lower Coal Measure Formation in the Gannister Horizon.

Cone

The diagrammatic reconstruction shown in fig. 9 will give an idea of the general form and structure of the cone. This reconstruction was based upon the twelve transverse sections. Each section was carefully traced on a blank sheet with camera lucida. One of these tracings is shown in fig. 1. After measuring each part of the cone traversed by the dotted line (*x*-*y*) across the longer diameter of the section, it was plotted on a graph sheet. A general survey of all the slides suggested a bent axis, for some of the sporangia were

cut transversely on one side of the axis and tangentially on the other. Only a bent axis as shown will fit the situation, taking for granted that the sections were cut parallel to each other from the block. As there were no longitudinal sections available from this same material, the diagrammatic longitudinal section of a sporophyll attached to the axis (fig. 14), together with those figured by WILLIAMSON and MASLEN, helped in roughly mapping out the form and relation of the various parts. As shown by the reconstruction, the general form is ovoid, measuring a little over 5.1 cm. in length and 2.4 cm. in diameter through the widest portion.

Cone axis

The axis is in excellent condition for an intensive study of the internal structure throughout its entire length. For this reason it would offer a good material for finding, as MASLEN suggested, "what variations in structure, if any, take place in different regions in the same cone."

The cone axis consists of a definite pith, a rather broad primary xylem bordered with protoxylem cells bulging out into points in several places around the xylem cylinder, a 1-2-celled meristematic region, and a narrow undifferentiated cortex (primary) containing sporophyll traces (fig. 2). Table I shows the dimensions secured from the slides, G 19 representing the lowest section, G 14 a middle section, and G 9 the second to the top slide. From this table it will be seen that: (1) the axis is long and slender, tapering to a point toward the top; (2) the pith is smaller at the bottom than at the top; (3) the primary xylem is thicker at the bottom than at the top; (4) the cortex maintains the same thickness throughout; (5) the meristematic region is very narrow, and maintains the same thickness throughout; (6) the protoxylem points number from 7 to 15 on each section, there being less in the top sections; (7) the sporophyll traces on each section are the same in number throughout the length of the axis.

Fig. 3 shows three stages in the separation of the sporophyll trace bundles from the peripheral protoxylem points. At *a* is a protoxylem point just beginning to separate, at *b* is another bundle with one or two meristematic cells inserted between the sporophyll

trace bundle and the xylem cells inside, and at *c* is a large one wholly separated from the xylem cylinder, but still imbedded in the cortex. On account of the absence of longitudinal sections it is impossible to give an account of the course of the sporophyll trace bundles through the cortex.

Sporophyll

Fig. 14 shows a reconstruction of the sporophyll bearing a sporangium on its adaxial surface. This reconstruction was based upon a series of sections (fig. 10) of a sporangium running through slides G 11–19. As in the former reconstruction, the whole was plotted on a graph sheet.

TABLE I

DIMENSION AND NUMBER OF PARTS OF CONE AXIS

Parts	Base G 19	Middle G 14	Top G 9
	mm.	mm.	mm.
Diameter of axis	1.28	0.95	0.53
Diameter of pith	0.25	0.26	0.30
Thickness of X^1	0.29	0.26	0.24
Thickness of meristem	0.002	0.002	0.002
Thickness of cortex	0.08	0.09	0.09
No. of protoxylem points	12	15	7
No. of sporophyll traces	9	9	9

As the sporophyll trace bundles leave the axis to form the pedicel, they carry with them an envelope of cortical parenchyma and assume a peculiar triangular shape (fig. 6 to fig. 10 *a*). The one corner turned toward the axis is blunt, while the outer two are sharper pointed. In the better preserved specimens may be recognized a row of epidermal cells. The greater part of the pedicel is taken up with thick walled parenchyma, leaving only a small area in the center for thinner walled parenchyma surrounding a simple bundle. This bundle consists of the same number (30–70) of elements as when they were in the cortex, and measures 11 mm. across. It has no definite protoxylem cells. In most of the pedicels there is a space on the dorsal side of the bundle which corresponds with MASLEN'S "phloem region." The thin walled parenchyma surrounding the bundle may be taken as a continuation of the meristematic region of the axis. In this connection may be mentioned the presence in some of the pedicels of a similar space on the ventral side of the bundle. Fig. 4

shows this space as the result of the breaking down of the fine parenchymatous tissue investing the bundle. From this it may be inferred that the dorsal space referred to as "phloem region" may have originally been fine parenchyma, as those surrounding the bundle, or some "secretory cells" claimed by SEWARD (**7**) to take the place of true phloem in *Lepidodendron*. Some of these spaces measure 0.25 mm. across, or occupy a larger area than the bundle. Whether this space represents a phloem, or not, we have the beginning of a collateral bundle here instead of in the cortex (**4**). From the way the sporophyll traces are arranged in the cortex the pedicels must have been spiral on the axis.

As represented in the diagram of a sporophyll (fig. 14), the pedicel rises with the axis at an oblique angle for a considerable distance before it swells up on the ventral side (fig. 10*b*). From this point up to a distance of 26 mm., the sporangium is attached on the adaxial surface of the pedicel, and inclines at about 20° angle with the axis. The pedicel at this region becomes winged and slightly keeled on the dorsal surface (fig. 10 *c*-*g*). Beginning with *d* there arise on the ventral side of both the expanding wings sclerenchymatous tissues, forming lateral cushions for the large sporangium (fig. 11*scl*). A striking peculiarity of this structure is the bilobed condition as the more distal end of the attachment of the sporangium to the pedicel is reached (fig. 15*scl*[1]). Fig. 11 at the right side shows the beginning of this bilobed condition.

Figs. 10 and 11 show that the pedicel sends out a distinct stalk to the sporangium, with a bounding layer continuous with the sporangium wall. Unfortunately none of the pedicel bundles at this region is cut transversely to show structure, but dark masses of the same size as those noticed lower in the pedicel indicate that there was no change in size or in form. Near and at the terminal ends of the wings are epidermal cells, but not as well defined as those figured by MASLEN. The "phloem space" could not be recognized in many of the sections, due perhaps to the crowded condition in this region.

As the periphery of the cone is reached, a broadened lamina rises upward from the widening distal end of the pedicel. This part tapers to a point high above its base. The actual length cannot be given, but, judging from the large and increasing number of lamina sections

as the apex is approached, and from the sporophyll reconstruction, it may measure 18 mm., thus justifying the specific name *foliaceus* given by MASLEN. A downward heel of the sporophyll lamina is represented in cross-section (fig. 1*h*) as triangular in shape. This is the only one identified as a downward heel; so it may be inferred that it is short.

The bundle at the base of the lamina (fig. 5) shows the same number of elements as previously noted. The elements here show scalariform markings, as they are cut slightly tangentially. A few fine parenchymatous cells surround the bundle, but outside these are scattered thick walled cells with scalariform thickenings. They are abundant on the ventral side, but absent on the dorsal side. These structures fit well the description of "transfusion tissue" or "short barred cells" (**9**). Toward the tip of the lamina the bundle becomes concentric, as the "phloem space" which accompanies it up to the region of the base of the lamina disappears as the apex is reached.

The striking feature of the species mentioned by MASLEN, the "indiscriminately scattered cells with dark contents" (or "secretory sacs"), is easily recognized. Fig. 8 shows the general distribution of these cells, while fig. 7 shows them in greater detail among the large celled parenchyma which makes up the greater part of the sporophyll lamina. Epidermal cells are recognized. Below these is a layer of hypoderma which is thicker along the middle of the lamina. Generally the ventral side has a thicker layer of hypoderma.

Fig. 12 shows the remains of a ligule between what apparently are extensions of the inner lobe of the lateral cushions beyond the sporangium stalk. Above the ligule is shown the sporangium wall wholly separated from the pedicel. This situation may be looked upon as though the ligule arises from a cavity under the projecting distal end of the sporangium, with the extensions of the inner lobe of the lateral cushions at the sides, and the ventral wall of the lamina on the outer side.

Sporangium

As already mentioned, the pedicel sends out a distinct stalk to the sporangium. This is continuous throughout the greater length of the pedicel, forming a longitudinal ridge of tissue between the sclerenchymatous cushions. This is sometimes known as the "sub-

archesporial pad." On top of this is laid a mass of sterile tissue. To this stalk is attached a large sporangium, the largest of which measures at least 27 mm. × 2 mm.

The wall of the sporangium consists of the ordinary palisade-like cells, thicker on the dorsal side just above the wings of the pedicel than on the ventral side. At places where the wall bends sharply, a layer of cellular tissue is often observed. This can be traced to the sterile mass of tissue laid on top of the subarchesporial pad. Arising from the ridge formed by the sterile mass of the tissue is a delicate radial plate, recalling the trabeculae structures in the modern *Isoetes*. Fig. 10 shows that toward the proximal end such a structure is absent, but as section *e* is reached there appears such a structure bifurcating near the top. Section *g* or the more distal sections show distinctly these trabeculae. A survey of a section of the cone from the axis to the periphery reveals the same situation. The basal and apical sections of the cone show no variation from this plan. From this it looks as though ontogenetic development of this structure must have begun from the distal end, and that these are definite plates partly dividing the sporangium case into three regions.

Spores

The numerous spores (fig. 13) which fill the sporangium measure approximately 0.032 mm. in diameter. On close observation the triradiate markings are noticeable on those not broken in the preparation. A thorough search of all the sections failed to disclose a single megaspore. In a way this result sounds startling, for, as it will be recalled, Mrs. D. H. SCOTT (**6**) in 1906 designated *Triletes diabolicus* as belonging to *Lepidostrobus foliaceus* Maslen. BERRY (**3**), however, says that the two kinds of spores may be produced upon different cones. The specimen under investigation is as perfect as can be expected. A glance at the reconstruction (fig. 9) will suggest the idea that although the lowest slide does not represent the real base of the cone, the specimen very nearly represents the whole cone. The converging of the general outline toward the base, the total absence of sporophyll lamina sections on the first two lowest slides (G 18–19), and the decreased number of sporangia sections on these support this view. The writer feels sure, therefore, that the specimen on hand is homosporous.

Related Lepidostrobi

On the basis of structure, the closest relatives of *L. foliaceus* Maslen are *L. Oldhamius* Will. (**1, 4, 8**), of which there are many forms, *L. Veltheimianus* (**5,** vol. 1), *L. Bertrandi* (**10**), *L. Binneyanus* (**1**), and *L. gracilis* (**1**). These, as well as *L. foliaceus* Maslen, agree in all the generic characters of *Lepidostrobus* as given by ZEILLER (**11**).

Diagnosis

The following characters are enumerated for the diagnosis of *Lepidostrobus foliaceus* Maslen:

1. Lower Carboniferous Formation; Gannister Horizon.

2. The cone is ovoid, measuring 5.1 × 2.4 cm.

3. The sporophyll has a long triangular pedicel (26 mm.) up to the attachment of the sporangium, and leaves the axis at an oblique angle of about 20°.

4. A distinct stalk to the sporangium elongated along the length of the pedicel is recognized; hence the sporangium is not sessile.

5. The pedicel under the sporangium and on the wings has two lateral sclerenchymatous tissues, each of which is bilobed at the distal end, seemingly acting as supports to the massive sporangium.

6. The ligules arise from a cavity overhung by the distal end of the sporangium.

7. On top of the subarchesporial pad is a ridge of sterile tissue from which arise sterile plates or trabeculae.

8. The sporangia bear only microspores; hence the specimen is homosporous. Another form (**6**) is reported heterosporous.

9. The axis consists of a small pith, a rather broad zone of primary xylem with peripheral protoxylem points, a narrow meristematic region, and an undifferentiated cortex imbedding sporophyll trace bundles.

10. The bundles consist of 30–70 scalariform elements, accompanied on the dorsal side with a "phloem space" beginning at the base of the pedicel as it leaves the axis, up to the region of the base of the sporophyll lamina where transfusion tissues appear.

11. The lamina of the sporophyll is composed mostly of large celled parenchyma, many cells of which show dark contents simulating "secretory sacs."

12. The upturned lamina is unusually long, attaining a length of 18 mm. in some cases.

From this diagnosis it is clear that the material studied is unmistakably identical with that partly diagnosed by MASLEN. It will be fair, therefore, to call it *L. foliaceus* Maslen. Fortunately the specimen is in excellent preservation to supply the needed structures in fine details, so that a full account of the species is now available.

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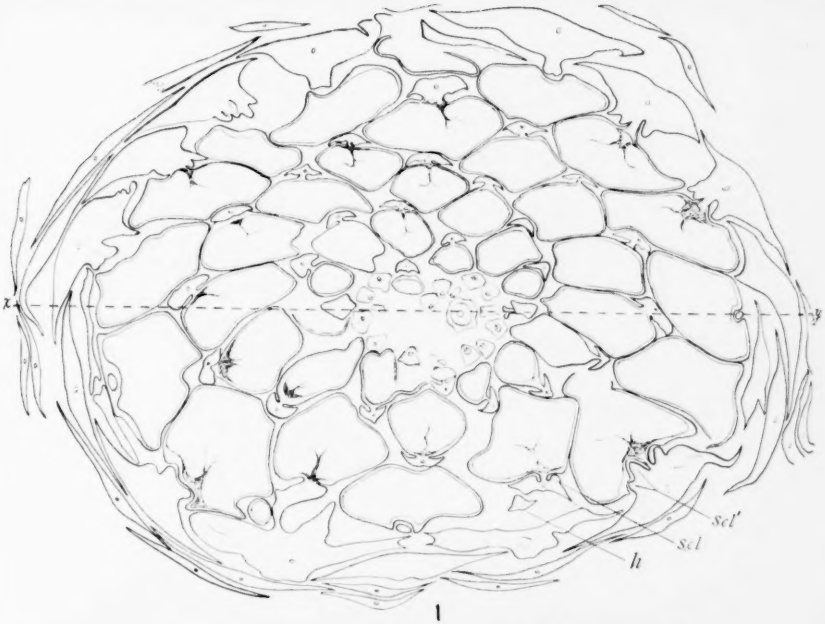
EXPLANATION OF PLATES XXX-XXXII

FIG. 1.—Transverse section through middle of cone (G 14), showing axis in middle surrounded with 17 pedicels, 2–3 rows of sporangia, and number of sporophyll lamina sections outside: *h*, heel of sporophyll lamina; *scl*, sclerenchymatous cushion; *scl*^{1}, bilobed sclerenchymatous cushion; ×4.

FIG. 2.—Transverse section of cone axis near base (G 18), showing narrow pith in center, rather broad primary xylem cylinder bordered with protoxylem



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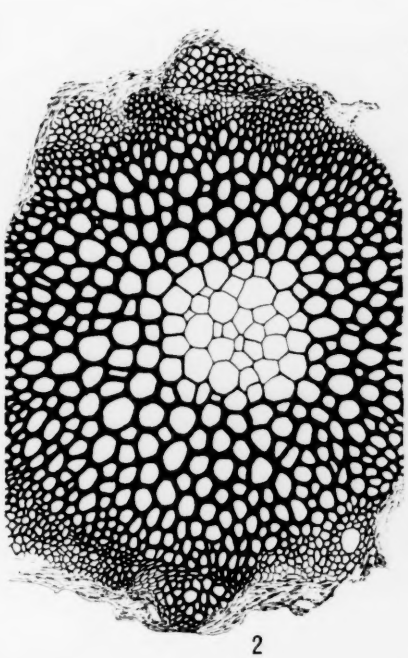

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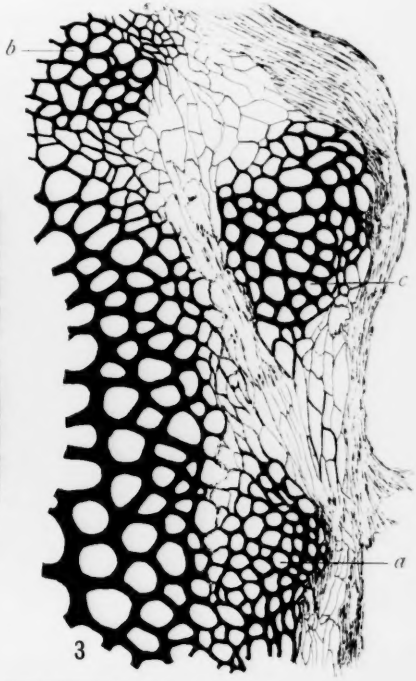
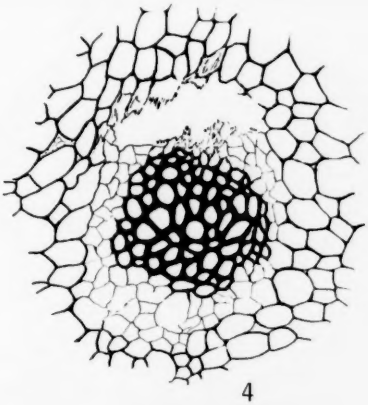


3

CAMPOS on LEPIDOSTROBUS



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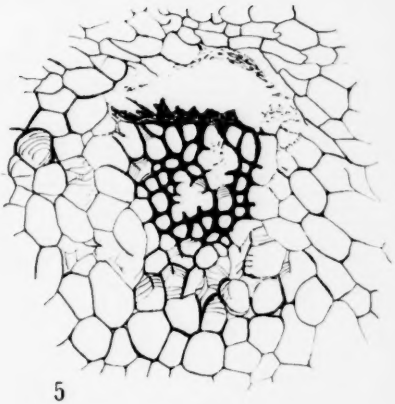

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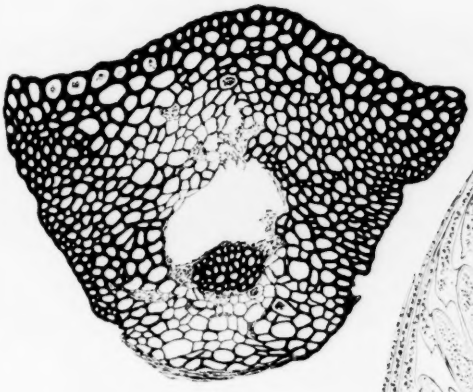

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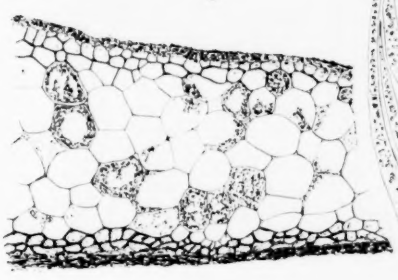

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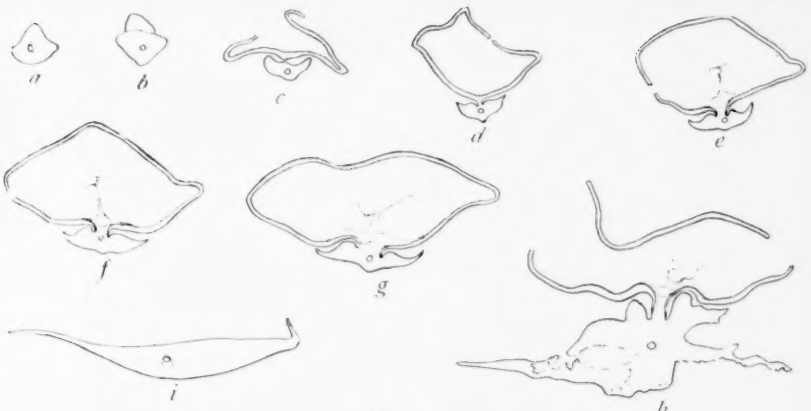
CAMPOS on LEPIDOSTROBUS



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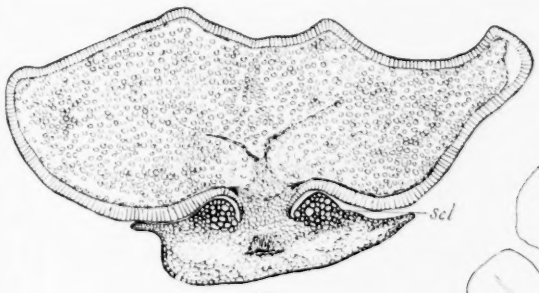

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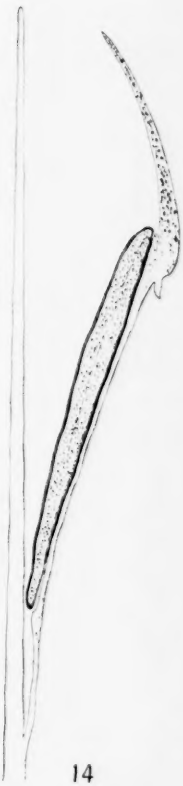

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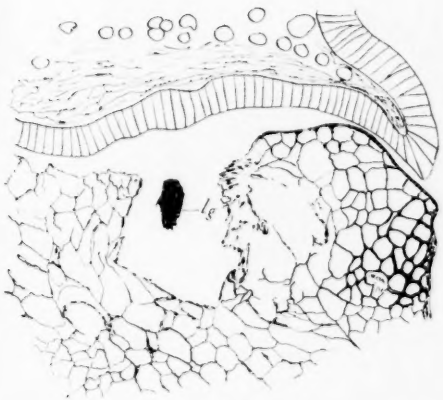

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13

CAMPOS on LEPIDOSTROBUS

points, 1-2-celled meristematic region, and narrow undifferentiated cortex containing sporophyll traces; ×73.

FIG. 3.—Details through outer portion of axis (G 18 at B), showing portion of xylem cylinder: *a*, protoxylem point just beginning to separate; *b*, bundle being cut off by insertion of meristematic cells; *c*, bundle wholly separated from xylem cylinder but still imbedded in cortex; ×196.

FIG. 4.—Details of pedicel bundle (G 11), showing on dorsal side (above) a phloem region, and thin walled parenchyma surrounding simple bundle; ×196.

FIG. 5.—Details of bundle (G 9 at E) at base of lamina, showing transfusion tissue on ventral side (below); ×196.

FIG. 6.—Transverse section of pedicel (G 18 at C) below sporangium attachment; "phloem space" above bundle; ×73.

FIG. 7.—Details of transverse section of sporophyll lamina (G 11 at C); epidermis partly disorganized but hypoderma well preserved; among large celled parenchyma are indiscriminately scattered cells with dark contents or "secretory sacs"; ×73.

FIG. 8.—Transverse section of sporophyll lamina (G 11 at C), showing general distribution of "secretory sacs"; ×21.

FIG. 9.—Reconstruction of median longitudinal section of whole cone based upon twelve transverse sections (G 8–19); ×2.

FIG. 10.—Series of sections of single sporophyll with sporangium (G 11–19): *a*, from G 19; *i*, from G 11; ×8.

FIG. 11.—Details of transverse section of pedicel and attached sporangium (G 9 at D); *scl*, sclerenchymatous cushion; ×21.

FIG. 12.—Transverse section of distal portion of sporangium and pedicel (G 10 at D), showing ligule (*lg*) between extensions of bilobed sclerenchymatous cushions; ×73.

FIG. 13.—Microspores showing characteristic triradiate markings; *a*, from G 8 or apex of cone; *b*, from G 14 or middle; *c*, from G 19 or near base of cone; ×363.

FIG. 14.—Reconstruction of sporophyll bearing sporangia adaxially (based upon series of sections sketched in fig. 10); sporophyll as shown inclines at 20° angle with axis; ×2.

BRIEFER ARTICLES

PARADICHLOROBENZENE IN THE HERBARIUM

The compound paradichlorobenzene, for which GUYTON and STEAR[1] have recently proposed the convenient and sensible abbreviation PDB, has lately come into extensive agricultural use as an agent for killing peach borers. It comes on the market in the form of volatile, whitish crystals giving off at ordinary temperatures a gas with a penetrating and pungent but not unpleasant odor, which is highly poisonous to insects. In this laboratory it has been tested for a year and a half in the mycological herbarium, and has been found to possess certain distinct advantages over other material used to protect fungi from insect attack. It has been found particularly valuable for disinfecting large boxes of specimens awaiting detailed study, and the large packages of material kept for class use, both of which are particularly likely to be attacked by insects because they are necessarily stored in such a way as to afford less protection than is given to regular herbarium specimens.

The gas is heavier than air; hence in disinfecting large boxes of material known or suspected to be infested with insects, the specimens are first placed in the box, then a small quantity of the PDB crystals is put on top in a folded paper or an envelope, and the box closed. When the space to be disinfected is small, the position of the crystals is unimportant. The specimens need not be removed from their envelopes unless time is pressing, in which case, if the specimens are unwrapped and a somewhat larger quantity of the crystals used, results will be secured more rapidly.

PDB is much more volatile than naphthalene, and will not entirely replace the latter for permanent use in the packets. In cold weather it has been found desirable to use the two together. A few crystals of PDB are added to the usual quantity of naphthalene placed in each packet; or, if a large number of specimens is being put up at one time, the two are mixed together in the proportion of one part of PDB to two or three of naphthalene, and a pinch of the mixture is put into each packet. It is not desirable to mix the two in hot weather, since the mixture melts at 40° C. and begins to cake at an even lower temperature, while the PDB alone remains solid up to 50° C. The compound is quite harmless to the specimens, and in the small quantities used seems to have no effect whatever upon human beings. It has been found so convenient and effective in the limited field in which it has been tried as to suggest that its use might well be extended to the protection of preserved specimens of other groups of plants.—G. W. MARTIN, *University of Iowa, Iowa City, Iowa.*

[1] Penna. Dept. Agric. 1924. General Bull. 383.

CURRENT LITERATURE

BOOK REVIEWS

Colloid symposium monograph

Attention of plant physiologists is called to the publication of the *Second colloid symposium monograph*,[1] which contains the papers presented at Northwestern University during the summer of 1924. There are twenty titles, some of which are worthy of note. Among these may be mentioned a paper by MICHAELIS on *The general principles of the effects of ions in colloids*, and one by FREUNDLICH on *Sols with non-spherical particles*. Several papers deal with emulsification. These are by HOLMES and WILLIAMS on *Polar emulsifying agents*, and *Iodine as an emulsifying agent;* and a paper by HARKINS on *The orientation of molecules in the surfaces of liquids*.

The outstanding contribution is a lengthy discussion of the prolamines and their chemical composition in relation to acid and alkali binding, by HOFFMANN and GORTNER. This excellent paper occupies about 150 pages, and constitutes what HOLMES calls "a masterly reply to certain implied criticisms of colloid chemistry found in LOEB's book on proteins and the theory of colloidal behavior."

Two types of combination between proteins, and acid and alkali, are recognized. Chemical combination holds between hydrogen ion concentration limits from P_H 2.5 to P_H 10.5, and between these limits the amount of acid or alkali bound depends on the chemical composition of the protein. When the acidity is greater than P_H 2.5, or alkalinity greater than P_H 10.5, however, an adsorption type of combination occurs; and at these extreme ranges all types of prolamines, regardless of their composition, bind approximately the same amount of acid or alkali.

Indications are found that the isoelectric "point" of a protein is really an isoelectric range, the position of the range on the P_H scale depending on the chemical composition of the protein. The *calculated* isoelectric points obtained by extrapolating the logarithmic curves for acid and alkali binding, however, are found to fall near the neutral point of water, P_H 7, which would be expected on the theory that at higher concentrations of acid and alkali, the combination follows the adsorption law. These calculated isoelectric points, therefore, are not related to the chemical composition of the protein. This paper deserves careful study by all who desire to keep abreast of our knowledge of the physical chemistry of the proteins.—C. A. SHULL.

[1] Colloid symposium monograph. Vol. II. Ed. by H. N. HOLMES. 8vo. pp. viii+368. N.Y.: Chemical Catalog Co.

MINOR NOTICES

Tree habits.—For the student of forest life who wants something more than a diagnosis of the species, a recent book by ILLICK[2] on tree habits will be welcomed. Those who desire to increase their ability to recognize the hardwoods at all seasons of the year may learn much from its pages. The writer seems to have attained a happy medium of style that lies between the dryness of mere taxonomic description and the sentimentality that characterizes some so-called "nature books." The book reads easily, while the information is scientifically accurate. The identification of the species is aided by illustrations, keys, and tables. In the latter are included many details of habit and structure that are seldom mentioned in textbooks, but are in continual use by ecologists, foresters, and lumbermen. The illustrations are numerous and excellent, including drawings of leaves, flowers, and fruits, and photographs of trunks, twigs, and entire trees. The glossary, keys, and index are adequate, although the last should include scientific as well as common names. While the book does not include all our broad-leaved trees, few species of wide distribution or of any economic importance are omitted. It is hard to conceive of a volume of this type that is likely to prove more companionable.—GEO. D. FULLER.

NOTES FOR STUDENTS

Temperature for ripening of sweet corn.—Not all are in agreement as to what the chemical composition of sweet corn should be at the time it is picked to be eaten, or to be packed into cans. Usually the sweetness is emphasized, but some state that the content of starch and crude fiber is of equal if not of greater importance. APPLEMAN and EATON[3] have determined the chemical changes in sweet corn during ripening, relating these to temperature, and attempting to make the results of some practical value as a guide for the picking of sweet corn in different parts of the country, or in different seasons in the same locality. The decrease in starch and the increase in sugar were the chief changes noted. At the time of greatest sugar content, the reducing sugars predominate, but these quickly decrease in amount. Since the reducing sugars are not nearly as sweet as cane sugar, the stage of greatest sweetness is not that of greatest total sugar content, but that of greatest cane sugar content. The changes in the amount of fat, crude fiber, and total nitrogen occurred early in the ripening process, and after that there was not much change in the amount of these constituents present. The rate of ripening is controlled by temperature. It took fifteen days for a late crop to reach the same stage of ripening that was reached by an early crop in six days. The temperatures of the two periods were compared, and after trying out several temperature indices, in an attempt to evalu-

[2] ILLICK, J. S., Tree habits: how to know the hardwoods. pp. iv+337. *figs. 196*. Amer. Nature Assoc., Washington, D.C. 1924. $4.00.

[3] APPLEMAN, C. O., and EATON, S. V. Evaluation of climatic temperature efficiency for the ripening processes in sweet corn. Jour. Agric. Res. **20**: 795–805. 1921.

ate the efficiency of temperature as the controlling factor in the ripening process, it was decided that the exponential indices furnished the best criterion. The time ratio for the ripening periods of the two crops was practically the same as the ratio of the averages of the daily exponential indices for the two periods. This ratio was 2.57, so the rate of ripening seems to follow the Van't Hoff-Arrhenius law. The exponential indices were used to predict for different localities and different seasons of the same locality the maximum number of days that sweet corn will remain in the best edible condition, as well as the approximate number of days required for corn to pass from the beginning of kernel formation to the stage of best quality for canning or edible purposes. These results should be of practical value to canners of sweet corn.—S. V. EATON.

The Sargasso Sea.—A recent report by WINGE[4] seems to add materially to our knowledge of this curious plant community. The vegetation is represented as varying much in abundance, from miles of open sea without a plant, through thin layers of drift, to rather dense masses of tangled plants. Doubt is expressed whether any of the drifting *Sargassum* belongs to anchored shore species. Eight floating forms or species are described and designated by the numbers I to VIII, three of these forming the great mass of the vegetation. It is suggested that these be transplanted to the shallow waters of the coast in order to determine their relation to coastal forms. *Sargassum* requires a water temperature of 18° C. in order to thrive, and this must be considered in experimental work.

The extent of the Sargasso Sea has been mapped, and it has been determined that its northern boundary varies seasonally, reaching farther north during the summer, dependent on the direction of prevailing winds. The other boundaries are relatively fixed.—GEO. D. FULLER.

Floral ontogeny.—THOMPSON[5] has contributed the first paper in a new series of botanical publications to be issued by the University of Liverpool. He states that "in the study of flowering plants attention has been so focussed on the adult organism that the facts of ontogeny have for the most part been passed unnoticed." In this initial study the floral ontogeny of the Amherstieae (Leguminosae) is described in great detail and well illustrated. The general purpose is to discover in this way suggestions as to relationships. As a result of this study of the tribe Amherstieae, the genera are rearranged according to the records of their ontogenies.

Floral ontogeny was first established by PAYER in 1857, but has been very

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[4] WINGE, Ö., The Sargasso Sea, its boundaries and vegetation. Rept. Danish Oceanographical Exp. 1908–10 to the Mediterranean and adjacent seas. **3**[2]: pp. 34. *figs. 14. 2 maps.* 1923.

[5] THOMPSON, J. M., Studies in advancing sterility. I. The Amherstieae. Univ. Liverpool, Publ. Hartley Bot. Lab. no. 1. pp. 54. *figs. 104.* 1924.

little developed since, although it is a subject well deserving cultivation. It is significant, therefore, that this field of research has been opened again in this series of studies to be published by the University of Liverpool.—J. M. C.

Trees and shrubs of Mexico..—STANDLEY[6] has published the fourth installment of the *Trees and shrubs of Mexico*, bringing together in organized form the known material of 37 families. This work has involved the study of 275 genera and 1738 species. Very few new genera and species are included, as the work is intended to assemble rather than to monograph the groups. One new genus (*Leucopremna*) of Caricaceae is established, and 43 new species, 11 of which belong to *Eugenia* (Myrtaceae). Much the largest family is the Cactaceae, since it is displayed more in Mexico than in any other region of the world. This family is presented by BRITTON and ROSE, and includes 54 genera and 523 species. The largest genera are *Neomammillaria* with 134 species, *Opuntia* with 87, and *Echinocereus* with 48. The other large families are Menthaceae (127 species), Asclepiadaceae (119 species), Solanaceae (114 species), and Melastomaceae (114 species).—J. M. C.

Single fertilization in barley.—HARLAN and POPE[7] have discovered some strains of barley in which certain abnormalities appeared in seed development, notably a few seeds lacking an embryo, and a much greater number deficient in endosperm. These seeds were investigated, and the conclusion reached that they were to be explained by failure of "double fertilization." No cytological investigation was undertaken, which might discover this variable behavior of the two "male cells."—J. M. C.

Embryo of Linum.—SOUÈGES[8] has published the results of his investigation of the embryogeny of *Linum catharticum*, with his usual fullness of detail and clear illustrations.—J. M. C.

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[6] STANDLEY, P. C., Trees and shrubs of Mexico (Passifloraceae-Scrophulariaceae). Contrib. U.S. Nat. Herb. **23**:849–1312. 1924.

[7] HARLAN, H. V., and POPE, M. N., Some cases of apparent single fertilization in barley. Amer. Jour. Bot. **12**:50–53. 1925.

[8] SOUÈGES, R., Développement de l'embryon chez le *Linum catharticum* L. Bull. Soc. Bot. France IV. **24**:925–938. 1924.

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